SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 15 December 2016

doi: 10.2903/j.efsa.2017.4690

Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms

EFSA Panel on Plant Protection Products and their Residues (PPR), Colin Ockleford, Paulien Adriaanse, Philippe Berny, Theodorus Brock, Sabine Duquesne, Sandro Grilli, Antonio F Hernandez-Jerez, Susanne Hougaard Bennekou, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Kyriaki Machera, Olavi Pelkonen, Silvia Pieper, Michael Stemmer, Ingvar Sundh, Ivana Teodorovic, Aaldrik Tiktak, Chris J. Topping, Gerrit Wolterink, Peter Craig, Frank de Jong, Barbara Manachini, Paulo Sousa, Klaus Swarowsky, Domenica Auteri, Maria Arena and Smith Rob

Abstract

Following a request from EFSA, the Panel on Plant Protection Products and their Residues developed an opinion on the science behind the risk assessment of plant protection products for in-soil organisms. The current risk assessment scheme is reviewed, taking into account new regulatory frameworks and scientific developments. Proposals are made for specific protection goals for in-soil organisms being key drivers for relevant ecosystem services in agricultural landscapes such as nutrient cycling, soil structure, pest control and biodiversity. Considering the time-scales and biological processes related to the dispersal of the majority of in-soil organisms compared to terrestrial non-target arthropods living above soil, the Panel proposes that in-soil environmental risk assessments are made at in- and off-field scale considering field boundary levels. A new testing strategy which takes into account the relevant exposure routes for in-soil organisms and the potential direct and indirect effects is proposed. In order to address species recovery and long-term impacts of PPPs, the use of population models is also proposed.

Keywords: in-soil invertebrates, microorganisms, effects, pesticides, protection goals, risk assessment

Requestor: EFSA

Question number: EFSA-Q-2011-00978

Correspondence: pesticides.ppr@efsa.europa.eu

Panel members: Paulien Adriaanse, Philippe Berny, Theodorus Brock, Sabine Duquesne, Sandro Grilli, Antonio F. Hernandez-Jerez, Susanne Hougaard, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Kyriaki Machera, Colin Ockleford, Olavi Pelkonen, Silvia Pieper, Rob Smith, Michael Stemmer, Ingvar Sundh, Ivana Teodorovic, Aaldrik Tiktak, Chris J. Topping, Gerrit Wolterink.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Non-target arthropod and In-Soil risk assessment: Peter Craig, Frank de Jong, Michael Klein, Ryszard Laskowski, Ton van der Linden (until July 2015), Barbara Manachini, Silvia Pieper, Robert Smith, Paulo Sousa, Ingvar Sundh, Klaus Swarowsky, Aaldrik Tiktak and Christopher J. Topping for the preparatory work on this scientific opinion, and EFSA staff Maria Arena and Domenica Auteri for the support provided to this scientific opinion.

Suggested citation: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, Adriaanse P, Berny P, Brock T, Duquesne S, Grilli S, Hernandez-Jerez AF, Bennekou SH, Klein M, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Stemmer M, Sundh I, Teodorovic I, Tiktak A, Topping CJ, Wolterink G, Craig P, de Jong F, Manachini B, Sousa P, Swarowsky K, Auteri D, Arena M and Rob S, 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j.efsa.2017.4690

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

The new regulatory framework for plant protection products (PPPs) laid out in Commission Regulation (EC) No 1107/2009 and Commission Regulation (EU) No 283/2013 and 284/2013 explicitly requires consideration of impacts on non-target species, on their ongoing behaviour and on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web. In view of this new legislative background and the new scientific developments, the European Food Safety Authority (EFSA) asked the Panel on Plant Protection Products and their Residues (PPR) to develop and update the guidance documents on terrestrial ecotoxicology (SANCO/10329/2002) under mandate M-2009-0002. The assessment of effects on biodiversity is not explicitly addressed under the existing guidance documents; appropriate risk assessment methodology therefore needs to be developed. This scientific opinion has been written as a precursor to the guidance document on the risk assessment for in-soil organisms. Other terrestrial organisms as previously covered in the SANCO Guidance 10329/2002, such as birds and mammals, non-target arthropods, bees and non-target terrestrial plants are covered in other EFSA scientific documents (EFSA, 2009a, 2013; EFSA PPR Panel, 2014a, 2015a).

In-soil organisms are species that dwell primarily in the soil and soil litter. In-soil organisms are exposed to plant protection products (PPPs) from contact and oral uptake routes of exposure in the surrounding soil compartment. A 'healthy' soil supports a range of ecosystem functions or services (such as nutrient cycling) that are essential for supporting the growth of crops as well as the organisms that depend on those crops. The working group of the PPR Panel reviewed the current environmental risk assessment, identified key drivers that sustain important in-soil ecosystem services in agricultural landscapes and developed proposals for specific protection goal (SPG) options for in-field and off-field areas. The SPG options will then be discussed and agreed in consultation with Risk Managers. The working group developed proposals for testing of effects as well as suggestions to calibrate the lower tier risk assessment steps.

The in-soil communities of invertebrates and microorganisms are the most diverse part inhabiting agricultural landscapes. Yet, the current risk assessment, at the first tier, examines a selection of invertebrate model species (e.g. *Eisenia fetida*/*andrei*, *Folsomia candida*/*fimetaria*, *Hypoaspis aculeifer*) and one microorganism-mediated process (N transformation). The currently requested tests were reviewed in relation to the proposed SPG options and the available data and the representativeness of the current standard species was discussed. The Panel suggests that the current test battery with the use of an appropriate (calibrated) assessment factor might cover the intra- and interspecies variability in toxicological sensitivity in soil, with the exception of some in-soil organisms when exposed via food and via litter. Note that the current trigger values as included in the Regulation 546/2011 have not been properly calibrated at the time of their inclusion in the Regulation. The Panel recommends adapting the test with *H. aculeifer* to take the uptake of contaminated food into account, and to develop a standardised test with isopods, to take exposure via the litter into account. For microorganisms, the Panel proposed retaining and advancing the N-transformation test, and adding a test with mycorrhizal fungi to the data requirements and risk assessment.

In a tiered approach, considering the possibilities for intermediate tier testing, the Panel acknowledges the usefulness of the Species Sensitivity Distribution (SSD) conceptual model (in intermediate tier A); however, standard SSD methodology cannot yet be applied to in-soil organisms until further guidance on how toxicity data can be combined (e.g. toxicity data for different taxonomic groups of in-soil organisms, etc.) will become available. Another option of intermediate tier can also be a microcosm study assessing effects on natural assemblages of in-soil communities (intermediate tier B), although further experience is necessary to apply this methodology in risk assessment. At higher tiers, the Panel recommends assessment of the response of communities of in-soil organisms to intended uses of PPPs, so that indirect effects on populations of key drivers can also be detected. Natural communities of in-soil organisms should be studied using field tests or semifield test like terrestrial model ecosystems (TMEs), pending on the context triggering the need for higher tiers.

The Panel recommends that species recovery and other long-term impacts (including multiple stressors) at the population level are best investigated using a combination of experimental data and population modelling, if these were including all relevant environmental and ecological parameters. However, since long-term impacts and indirect effects at the community level cannot be assessed using population models, (semi)field studies with natural assembled communities are needed. It should be noted that recovery of populations of soil organisms and soil processes after impact of PPP intended uses might be demonstrated only later than the proposed time scale of tolerable effects in

the SPG options. For example, if effects should not persist for more than 6 months, key drivers may need to be monitored for a longer time period depending on their generation time to exclude effects on reproduction and indirect effects persisting more than 6 months.

It is suggested that assessment factors be derived on the basis of statistical modelling of the relationships between effects for different species in the various possible lower tier tests and higher tier field studies and the surrogate reference tier. In particular, a Bayesian network model can exploit information from experimental data and from expert judgement in the absence of suitable data. Such a model provides a relatively transparent method for deriving assessment factors in order to ensure a high probability of acceptable effects for uses that pass the risk assessment.

Further research needs such as standardisation of additional testing protocols, development of a range of representative scenarios and models of relevant taxa for population modelling, use of toxicokinetic/toxicodynamic (TK/TD) for in-soil organisms, etc. have been identified.

Table of contents

1. Introduction

In 2008, the PPR Panel was tasked by the European Food Safety Authority (EFSA) to revise the guidance document (GD) on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final) (European Commission, 2002), which is currently used in the routine environmental risk assessment for terrestrial non-target organisms (except for birds and mammals and non-target arthropods) exposed to active substances in plant protection products (PPPs). The replacement of Directive 91/414/EEC[1] by Regulation (EC) No 1107/2009[2] (hereafter referred to as 'the Regulation') in June 2011 called for revision of the existing GD in order to include new elements in environmental risk assessment (ERA), e.g. cut-off criteria and protection goals.

It was decided to split the task and to address separately the risk for different groups of organisms, i.e. in-soil organisms, non-target arthropods (NTAs), amphibians and reptiles, and non-target terrestrial plants (NTTPs). For each group of organisms, the PPR Panel first summarises the science behind the respective risk assessment in a scientific opinion and, in a second step, EFSA will develop practical guidance on how to perform the risk assessment. The present Opinion is focussed mainly on in-soil invertebrates and soil microorganisms. Vertebrates such as moles are dealt with in the Guidance for birds and mammals (EFSA, 2009a). Rooted plants are dealt with in the Opinion on non-target terrestrial plants (EFSA PPR Panel, 2014a). Algae are also not covered in the present Opinion because they do not seem to play a key role in the majority of the agricultural soils.

For the purpose of this Scientific Opinion and for consistency with the definitions as given in the recent Opinion of the PPR Panel on NTAs (EFSA PPR Panel, 2015a), in-soil organisms are defined as species that dwell primarily in the soil and soil litter layer. In-soil organisms may become exposed to PPPs from contact and oral uptake routes taking place in the surrounding soil compartment (EFSA PPR Panel, 2015a). The opinion is concerned with all non-target in-soil organisms, meaning all those in-soil organisms that are not indicated as the target pest species an active substance and PPP are effective against.

According to ISO 11074:2005, soil is defined as the upper layer of the earth's crust transformed by weathering and physical/chemical and biological processes. It is composed of mineral particles, organic matter, water, air, and living organisms organised in generic soil horizons. Soil performs a multitude of key environmental, economic, social and cultural functions, and could be regarded as the most complex biological environment directly affected by PPPs. Soil, for example, provides food, biomass and raw materials and plays a central role as a habitat and gene pool (biodiversity). In-soil organisms, including macro-, meso-, microfauna and microorganisms, are extremely diverse and contribute to a wide range of ecosystem services, such as nutrient cycling, pest and disease control, natural attenuation of pollutants, soil formation and stabilisation, etc. All these important ecosystem services could potentially be impacted by the intentional release of PPPs in the environment if the key drivers of the services were to be adversely affected by exposure to pesticides.

General protection goals are stated in the European legislation but are not precisely defined. A precise definition is however crucial for designing appropriate risk assessment schemes. Therefore, specific protection goal (SPG) options are presented, to be used in consultation processes with risk managers and stakeholders. It is the responsibility of risk managers to select the final SPG options that should be addressed in decision schemes of guidance documents. According to the PPR Panel Opinion (EFSA PPR Panel, 2010a), different groups of in-soil organisms (earthworms, microarthropods, macroarthropods, nematodes, gastropods, mycorrhizae and other fungi, bacteria, etc.) have been identified as providers of important ecosystem services in the soil ecosystem. SPGs have been developed considering six dimensions, namely ecological entity, attribute, magnitude of effects, temporal scale of effect, spatial scale of effect and degree of certainty.

Proposals for SPGs have been defined both for in-field and off-field as in-soil organisms also occur and are potentially exposed to PPPs outside the treated field. However, dispersal ability and biological characteristics need to be considered in the context of the pesticide risk assessment for these organisms. Some organisms move between fields and across field boundaries, so that recolonisation processes from the off- to the in-field might take place in relevant time frames. Others move only a limited distance within a field and might predominantly recover from PPP effects by processes that govern internal recovery. Considering the time-scales and biological processes related to the dispersal of the majority of in-soil organisms compared to terrestrial non-target arthropods living above soil, the

[1] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

[2] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Panel proposes that in-soil environmental risk assessments are made at local scale, considering processes at the field boundary scale. Unlike NTA, 'action at a distance' is not expected to be important for most in-soil organisms. Recovery by recolonisation would be important at very long temporal scale, and thus the landscape-level assessment is not needed.

In contrast to human toxicology, where individual health is protected by studies on several surrogate species, ecotoxicology is based on testing a limited number of species to make inferences about a much larger number of species. ERA for in-soil organisms is at the extreme end of the RA spectrum, because the diversity of in-soil species is much greater than for any other group of organisms in an environment directly affected by PPPs, while the tests and test data available are few.

This Opinion is structured to address key scientific aspects behind ERA for in-soil organisms, with a focus on in-soil invertebrates and microorganisms, including the major points resulting from the EFSA public consultation on the SANCO Guidance Document on Terrestrial Ecotoxicology. After a brief explanation on how the risk assessment for in-soil organisms is currently done, a section on the further elaboration of the proposed SPGs option is presented. Also, a general framework with an overview on the key aspects on the possible future risk assessment scheme for in-soil organisms is presented, followed by discussion on aspects of both exposure and effects assessment. For exposure of in-soil organisms, the focus is on the work developed by EFSA for spray application to annual crops under conventional or reduced tillage (development of exposure assessment for permanent crop is on-going and an update of the EFSA GD 2015 is foreseen by the end of 2017). On the effects-assessment side, existing and promising testing approaches are presented including tests for intermediate and higher tiers, how to deal with persistency and how to tackle recovery. These approaches may possibly be adopted in the future ERA scheme.

1.1. Background and Terms of Reference as provided by the requestor

In view of the revision of the current risk assessment for terrestrial organisms, in 2008, EFSA launched a public consultation on the SANCO Guidance Document on Terrestrial Ecotoxicology (EFSA, 2009b).

The aim of the public consultation was to collect issues and gaps identified by different stakeholders to be used as inputs in the revision of the terrestrial guidance.

A total of 33 comments were received from different stakeholders on the chapter about in-soil organisms (Chapter 6 of the SANCO guidance). The main comments concerned the following:

- Development of specific protection goals for in-soil organisms
- More clarity on the level of assessment (structure vs function)
- More guidance on persistent substances
- More guidance on how to consider bioavailability when interpreting effect test results and need for more standardised test design (% peat, addition of feed, application of the test item, correction factor)
- Earthworm field studies: more guidance on the evaluation of effects and acceptability criteria (% effects based on total earthworm numbers, biomass, safety factor, etc.). The use of the guidance on how to summarise earthworm field studies was suggested.
- Introduction of semifield tests (e.g. terrestrial model ecosystem (TME))
- More guidance on the interpretation of effects on soil microorganisms
- More guidance on the exposure assessment (measurement of the concentration in the test, selection of the appropriate predicted environmental concentration (PEC), persistence, etc.).

1.2. Terms of Reference as provided by EFSA

EFSA tasked the Pesticides Unit and the PPR Panel on the following activity, taking into consideration the legislative background, stakeholder comments as reported in Section 1.1 and the recommendations and priorities identified by Member States.

Development of Guidance on risk assessment for in-soil organisms, with the following deliverables:

- Opinion addressing the state of the science to be delivered by the PPR Panel by April 2017;
- Public consultation on the draft Opinion of the PPR Panel to be issued by the 1st quarter of 2016;
- Guidance of EFSA to be delivered within 2 years after the agreement with risk managers on the specific protection goals;
- Public consultation on the draft Guidance of EFSA.

1.3. Legislative Background

Active substances used in plant protection products (PPPs) are approved in the European Union (EU) under Regulation (EC) No 1107/2009. The Regulation requires that 'substances or products produced or placed on the market do not have any harmful effect on human or animal health or any unacceptable effects on the environment'. With respect to the environment, this includes, in particular, considerations of the impact on non-target species, including the ongoing behaviour of those species, and the impact on biodiversity and the ecosystem.

New Commission regulations laying down the data requirements for the dossier to be submitted for the approval of active substances contained in PPPs and the authorisation of PPPs (Commission Regulation (EU) No 283/2013[3] and 284/2013[4]) were published in 2013. Those documents provide information on the core data needed to assess active substances and PPPs. As a general requirement for substance approval, it is stated in Commission Regulation (EU) No 283/2013 that 'the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall be considered'.

Active agents as well as formulated products containing active agents (microbial PPPs) have specific data requirements and they are not specifically addressed in this opinion.

2. Current risk assessment

2.1. Current risk assessment for in-soil organisms and other background documents

The state of the art regarding the risk assessment of pesticides to in-soil organisms is presented in this chapter. In particular, an overview is given on the 1) current risk assessment approaches according to the SANCO/10329/2002 Terrestrial Guidance Document (European Commission, 2002); 2) background documents, such as workshop on semifield methods for the environmental risk assessment of pesticides in soil (PERAS workshop, Coimbra, 2007; Schäffer et al., 2008, 2010) and guidance for summarising earthworm field studies (De Jong et al., 2006).

2.1.1. Terrestrial Guidance Document SANCO/10329/2002

The current risk assessment for in-soil organisms is carried out according to the SANCO/10329/2002 Terrestrial Guidance Document developed under the Council Directive 91/414/EEC**.** This Directive was repealed in 2009 by the (EC) Regulation 1107/2009, while the Commission Regulations (EU) No 283/2013 and 284/2013 laid down the new data requirements for active substances and plant protection products (PPPs), respectively. Therefore, only the parts of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) covered by the regulations will be considered in the following paragraphs.

The risk assessment for in-soil organisms follows the principle of the risk assessment paradigm: 1) hazard identification, 2) hazard characterisation, 3) exposure assessment and 4) risk characterisation.

A tiered approach is used. The concept of the tiered approach is to start with a simple, conservative assessment and to go towards more complex evaluations (higher tiers), when necessary.

2.1.2. Exposure assessment

The exposure characterisation is represented by a comprehensive evaluation of fate and behaviour of the active substance and transformation products in soil of the treated area, including the estimation of PECs. The initial PECs values after single or multiple applications and PECs plateau are calculated according to FOCUS (FOCUS, 1997). The choice of the relevant PECs to be used for risk assessment will depend on the characteristic of the active substance (e.g. persistence in soil) and on the intended uses.

[3] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–94.

[4] Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 85–152.

2.1.3. Effect assessment

The effect assessment is represented by a comprehensive investigation of the dose–response relationships, in order to derive toxicity endpoints (e.g. LC_{50}, NOEC), which can be compared with the predicted environmental concentrations. According to the new data requirements, the studies listed below should be conducted and reported unless it is proven that the contamination of soil is unlikely. It is highlighted that the acute toxicity study on earthworms is no longer a data requirement.

- Test for sublethal effects on earthworms (*Eisenia fetida* or *Eisenia andrei*). The test is conducted according to the OECD guideline 222 (OECD, 2004) and information on the effects on growth, reproduction and behaviour of the earthworms should be reported. The relevant endpoint might be either EC_{10} or EC_{20} to be presented together with a NOEC.
- Test on springtail *Folsomia candida* (OECD, 2009) and mite *Hypoaspis aculeifer* (OECD, 2008) for PPPs applied directly to soil as soil treatments. For PPPs applied as a foliar spray, data on soil invertebrates other than earthworms may be required in case concerns have been identified in the risk assessment of non-target arthropods, as data on both the hymenopteran parasitoid *Aphidius rhopalosiphi* and predatory mite *Typhlodromus pyri* may be used in an initial risk assessment. The relevant endpoint might be either EC_{10} or EC_{20} to be presented together with a NOEC.
- Test on the impact of active substances and PPPs on soil microbial activity in terms of nitrogen transformation (OECD, 2000). The test is done at two concentrations, the PEC (= maximum predicted concentration in soil) and a multiple of the PEC as the worst case. The results are reported as the ratio of the nitrate-formation rates at the PEC relative to the control, expressed as a percentage of the control rate.

In case further refinements of the risk are triggered, field studies reflecting the intended uses of the PPP, the environmental conditions likely to arise and species that will be exposed, should be conducted, as indicated in the Uniform Principles (Commission Regulation (EU) No 546/2011). Field studies evaluate the effects on abundance and biodiversity, taking into consideration the likely level of effects, the species/groups affected, population recovery (within 1 year) as well as information on the application and fate of the PPP (EPPO, 2003). However, at present, there are few standardised higher tier protocols. The litter bag test is one example mentioned in the terrestrial SANCO guidance document but this is more concerned with functional rather than structural endpoints.

The risk to in-soil organisms other than earthworms can be further refined using a more realistic test substrate or exposure regime.

2.1.4. Risk assessment

The risk characterisation is represented by the calculation of appropriate risk quotients. For earthworms and other soil macroorganisms, SANCO/10329/2002 recommends calculating the acute and chronic toxicity exposure ratios (TERs). Only the chronic TER would be currently relevant, however, based on the new data requirement.

TERs are compared with trigger values defined in the Uniform Principles (Commission Regulation (EU) No 546/2011[5]) to establish whether the risk is low (acceptable) or high (unacceptable). Triggers are sometimes described as 'safety factors' that should take into account uncertainties in the intra- and interspecies variability and the extrapolation of toxicity endpoints from laboratory to field (including uncertainties with regard to the actual exposure in the field). For earthworms and soil macroorganisms, the current trigger value is 5. If the TER values are below 5, a high risk is identified. For soil microorganisms, the magnitude of effects is directly assessed in terms of risk. According to the Regulation 546/2011, a low risk to microorganisms is demonstrated if the percentage of effect is below 25% after 100 days.

[5] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

2.2. Other background documents

Among the background documents used for drafting, the present Scientific Opinion, the Panel considered:

a) The outcome of the PERAS workshop (Schäffer et al., 2008, 2010) aiming at identifying suitable semifield tests able to detect effects of PPPs on in-soil communities to be used in a tiered approach of pesticides risk assessment. Further information on semifield tests are given in Section 9.

b) The guidance for summarising, reporting and evaluating field studies with earthworms (De Jong et al., 2006) developed by the Dutch platform for the assessment of higher tier studies. The document did not provide guidance on the use of the results in risk assessment. In the current practice, studies done according to several protocols can be submitted as part of a dossier for approval of active substances. For field studies, the ISO guideline (ISO, 1999) and the papers by Greig-Smith et al. (1992) and Sheppard et al. (1998) are cited in the SANCO terrestrial guidance. When an earthworm field study is included in a dossier for pesticide authorisation, the rapporteur Member State (RMS) has to make an evaluation report in which the data should be summarised in a concise and transparent way and the validity has to be discussed. According to De Jong et al. (2006), the reliability of the test should be evaluated by assigning a Reliability Index (Ri). The reliability scale goes from fully reliable (Ri1) to reliable with restrictions (Ri2) and not reliable (Ri3). The studies considered as not reliable are not used in risk assessment. It has to be remembered, however, that a reliable field study might not always be relevant for risk assessment. Different items for the description of field studies (e.g. purity of the substance, test site, mode of application, dosage, test design, sampling, etc.) and the reporting of results (e.g. actual concentration, type of endpoint, statistical comparison, etc.) are proposed to be checked when summarising and evaluating field studies with earthworms and a decision on reliability can be reached after checking all the recommended items.

3. In-soil organisms

3.1. In-soil organisms in the scope of this Opinion

In-soil organisms are broadly separated into two groups: soil fauna and microorganisms (this division is also endorsed in this opinion). Soil microorganisms are a very diverse group of organisms that are generally not visible to the unaided eye (< 100 μm body size). The major groups of soil microorganisms are bacteria, archaeans, fungi and protozoa, and they are unified by the lack of ability to form distinct tissues or organs. They operate on a spatial scale of a few millimetres and their generation time goes from hours to a few days. Soil fauna includes diverse organisms such as nematodes, potworms, earthworms, mites, springtails, beetles, ants, and termites. Fauna can be further divided by body size into macrofauna (> 10 mm length, > 2 mm width; e.g. earthworms, millipedes, centipedes, woodlice, termites, ants, beetles); mesofauna (0.2–10 mm length, 0.1–2 mm width; e.g. microarthropods and potworms) and microfauna (< 0.1 mm length, < 0.1 mm width; mainly nematodes) (see Figure 1). For further information on the biology of in-soil organisms which are in the scope of this Opinion, please see Appendix A.

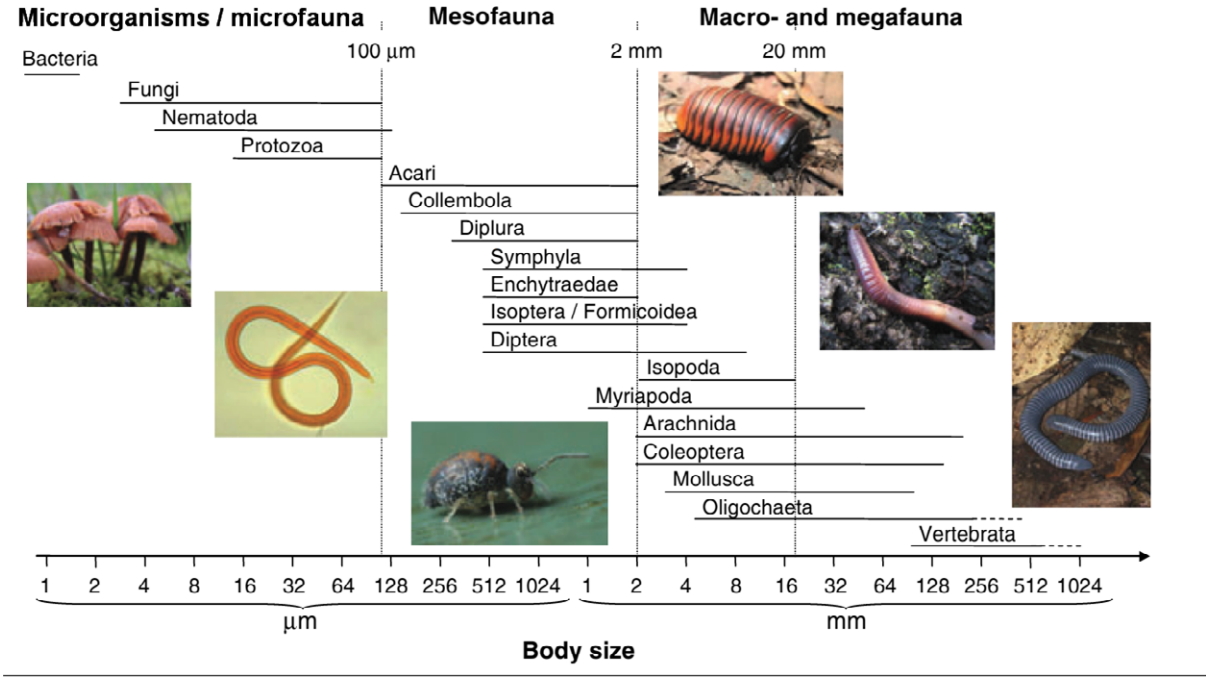

Figure 1: Representation of the main taxonomic groups of soil organisms on a body-width basis (Reprinted with permission from John Wiley and Sons after Swift et al., 1979) from Decaens (2010) and Barrios (2007) (all photo credits: Flickr, http://www.flickr.com/)

3.2. Dispersal, recovery potential of in-soil organisms

Population recovery of in-soil organisms can be internal and/or external. Internal recovery depends upon surviving of individuals in the stressed ecosystem or upon a reservoir of resting propagules (e.g. seeds and ephippia) not affected by the use of pesticides or other environmental stressors. In contrast, external recovery depends on the immigration of individuals from neighbouring areas by active or passive dispersal. Species life-history traits are considered key elements in determining the rates of recovery of affected population. Important species life-history traits are the number of generations per year and related life-history strategies (r-K), the presence of relatively insensitive (dormant) life stages and the capacity of organisms to migrate actively from one site to another. For example, voltinism (pertaining to the number of broods or generations per year) may be an important trait determining rates of population recovery of invertebrates (EFSA Scientific Committee, 2016a).

> 'Dispersal is a central ecological process that allows colonisation of new habitats and exploitation of spatially and temporally variable resources (..). Active dispersal of animals (as opposed to passive dispersal, where individuals could be transported by an external agent and has not necessarily a cost for the individual) is the result of three successive behavioural stages. It involves the departure from a breeding site, crossing to a new place, and settlement. It can occur at any life stage, at any spatial scales above the individual range and within more or less heterogeneous landscapes (...). It is assumed to depend on the balance between the costs and benefits of dispersal (...), which are strongly determined by both environmental conditions (e.g. habitat quality, habitat fragmentation, patch size, density, predation) and individual life traits'. (Caro et al., 2013a)

3.2.1. Potential for internal recovery

Macrofauna

As mentioned above, internal recovery depends upon the reproduction capacity and it is, therefore, linked to the generation time of a species. Thus, information on the life-cycle of species is considered crucial for understanding the recovery potential after toxic effects due to pesticides application. Earthworms have been proven to produce eggs during the whole year. The eggs are contained in cocoons. If the soil is too dry, the cocoons are deposited deeper into the soil. Biomass and size of

earthworm populations might be influenced by many parameters, including cocoons production which can be affected by seasonal variation in soil moisture, temperature, food supplies and other environmental factor, although earthworms can potentially produce cocoons throughout the year (Edwards and Bohlen, 1996).

It is well reported in the literature that few cocoons are produced in the winter period while the highest number is produced in the period May–July. Generally, the number of cocoons decreases with decreasing temperature, but the relationship is different for different species since the influence of environmental factors on population dynamics differs among earthworms of different ecological categories. Epigeic[6] earthworms, living and feeding mainly on the litter layer, may be more affected by seasonal temperature variations than endogeic[7] or anecic species[8] (those that inhabit permanent or semipermanent burrow systems in the soil) (Monroy et al., 2007).

Venter and Reinecke (1988) attributed to the availability and the quality of food as well as the maintenance of optimal moisture conditions a great importance for the growth rate of the *E. fetida* at 25°C. As shown in Appendix B, E. *fetida* displays in comparison to other earthworm species a relatively short life cycle with a high reproductive rate. Appendix B lists the life-cycle parameter for 12 Lumbricidae species. Total time for development ranges from 38 to 74 weeks.

Most land snails are oviparous and lay their eggs in clutches at sheltered places (e.g. soil cracks or burrows, under stones, among herbage (Barker, 2001). The number of eggs laid per clutch is highly variable within but also between species. According to Barker (2001), small terrestrial gastropods show a particular low fecundity, tending to produce only few eggs throughout their life (e.g. six in *Punctum pygmaeum* (Draparnaud) (Punctidae) during an average life span of 170 days). Larger animals may deposit several egg clutches per season (Kerney et al., 1983) and there often is considerable variation in the number of eggs per clutch and size of eggs within species, depending on the size and age of the parent animal, but also on environmental factors such as competition, and seasonality in climate (Barker, 2004). Mortality during the early life stage of terrestrial gastropods is rather high and it is not unlikely that only 5% or fewer animals of one egg clutch reach sexual maturity. Many terrestrial gastropods reach sexual maturity after 1 year, while the largest terrestrial gastropod species (but also some small species belonging e.g. to the genera *Columella* and *Vertigo*) may take 2–4 years to reach sexual maturity (Kerney et al., 1983).

Overall, it is concluded that especially smaller terrestrial gastropod species that make up the greater part of terrestrial gastropod diversity (see e.g. Sturm et al., 2006) are likely to have a poor recovery potential due to their low number of produced offspring and also their generation time may be rather long.

Most temperate species of isopods are seasonal breeders. However, there is a large variation in the period and duration of the breeding season. While some species breed in spring, others breed during the fall. Most species from temperate and Mediterranean habitats have breeding seasons lasting 4–8 weeks (Warburg et al., 1991), while others from tropical or temperate regions, the breeding season may last 3–6 months (Warburg, 1993). This is seen in tropical species *Orodillo maculatus* and subtropical species *Bethalus pretoriensis* that present a breeding season longer than for temperate species. The species *Porcellionides pruinosus*, however, represents an exception since it can breed continuously in tropical and temperate habitats.

Many woodlice species are iteroparous between years, i.e. they can produce more than one brood per year. Females of *Porcellio scaber*, for example, can produce up to three broods per year, whereas the species *Porcellio laevis* can produce up to six broods. This can vary also with the age of the individuals, and climate. In a warmer climate (California), first year females of *Armadillium vulgare* produce one brood within a year, while second year females can produce two broods. However, in East Anglia, the same species was semelparous. Fecundity in woodlice is associated with body size (Alikhan, 1995). Larger *A. vulgare* females can produce two broods per seasons, compared to one brood of smaller females.

The number of broods during a female life time can vary across species, going from one brood in certain populations of *A. vulgare* and other Armadillidiidae to more than six in populations of the species *P. pruinosus* and *P. laevis*.

[6] Epigeic earthworms live within the litter layers.
[7] Endogeic earthworms are unpigmented geophagous worms that live and feed within the soil' (Lavelle and Spain, 2005).
[8] Anecic earthworms feed on surface litter that they mix with soil but pass most of their time in subvertical subterranean galleries created within the soil' (Lavelle and Spain, 2005).

Climatic parameters, such as temperature, can influence reproduction. Increased temperature shortened the development time for mancas[9] of *Oniscus asellus* and accelerated the reproduction in *A. vulgare* (Warburg, 1993).

Mesofauna

Enchytraeidae species can reproduce either by sexual reproduction or asexually. For species able to reproduce sexually, adults lay cocoons that are a sort of mucilaginous bag, containing from 1 to 48 eggs. Hatching rate is usually high and can range from 19% to 97%. Enchytraeidae can produce 4–10 immatures per adult per year with a developmental period of 4–12 months in British meadow. A total life cycle in the range of 60–120 days from cocoon hatching to maturity has been reported under optimal conditions (Lavelle and Spain, 2005).

Westheide and Graefe (1992) reported life-cycle data for two species of Enchytraeids: *Enchytraeus crypticus* and *Enchytraeus doerjesi*. Burgers and Raw (2012) reported data from two sources on *Enchytraeus albidus* (see Table 1).

Table 1: Life-cycle data on three species of Enchytraeidae (sexual reproduction)

Species	Embryological development (days)	Hatching to maturity (days)	Total life span (days)	Cocoon production (d^{-1})	No eggs in a cocoon	Mean No eggs (d^{-1})
Enchytraeus crypticus	9.06[(a)]	8.3	81.6[(a)]	0.62	7.6	4.6
Enchytraeus doerjesi	6.8	8.5	93[(a)]	0.9	5.1	4.3
Enchytraeus albidus	–	44.5/21	68.3/261	0.22/0.40	4–5/1–35	

(a): Average of different values obtained for populations originating from different localities.

Asexual reproduction of Enchytraeidae occurs through fragmentation of individuals to form a few new ones, see Table 2.

Table 2: Life-cycle data on two species of Enchytraeidae (asexual reproduction)

Species	No of fragments	Development time to a complete worm (days)	Reference
Enchytraeus fragmentosus	3–14	10	Lavelle and Spain, 2005
*Cagnettia sphagnetorum**	2–3	8–26	Lavelle and Spain, 2005

*: This species starts to fragment when individuals have more than 42 segments.

According to species and size, Collembola species can have a number of stages going from 4 to 50. Development through the reproductive instars can take 40 to 400 days and moulting occurs continuously over the entire life (Lavelle and Spain, 2005). The fecundity of collembolan females depends on the number of eggs laid in each clutch and the total number of clutches produced. A female of the species *Sinella curviseta* and *Willowsia jacobsoni* can produce an average of eight clutches with 50 eggs each during the entire life-cycle, under laboratory condition and with continuous access to a male. Overall, collembolan species have been reported to lay 100–600 eggs during the entire life time, which is around 1 year (Lavelle and Spain, 2005).

Embryo development takes about 10 days for the species *Tomocerus ishibashi*. For the species *Entomobrya nivalis*, egg development has been reported as taking 25 days at 9°C, 15 days at 13°C and only 7 days at 20°C.

The maximum life span for a springtail under controlled conditions is 5–7 months for the species *Pseudosinella impediens*. However, under realistic conditions, some species can live longer, especially in stable cave environments. The complete life cycle of *Cryptopygus antarcticus* may take from 2 to 7 years, since in very cold climates growth and reproduction are much slower.

Some species are univoltine while other can be multivoltine. For example, the species *Tomocerus cuspidatus* is univoltine with a short breeding period in spring, while the species *Entomobrya aino* is multivoltine.

[9] Young isopod crustaceans hatch directly into a manca stage, which is similar in appearance to the adult, but they lack the seventh pair of pereiopods. They undergo progressive moults of manca stages, two in general, until the complete development of the seventh pair of pereiopods and the beginning of the development of secondary sexual characteristics (Brum and Araujo, 2007).

Fountain and Hopkin (2005) reported for *Folsomia candida* an average life span for females of 240 days and 111 days at 15°C and 24 °C, respectively. The number of eggs laid by a female can decrease from 1,100 to 100 going from 15 to 27 °C. An adult female may go through 45 moults in her lifetime with short reproductive instars (1.5 days) alternating to longer non-reproductive periods (duration 8.5 days).

At 20°C, the average duration of the five juvenile instars is 3 days for *F. candida* and maximum 4 days for *F. fimetaria*. Sexual maturity is attained in the 6th instar occurring around age 15–16 days for *F. candida* and a few days later for *F. fimetaria*. Egg development for *F. fimetaria* took 9.5 days, hence similar to 9–11 days observed for *F. candida*. Reproduction may be parthenogenic or bisexual. Generally, it is reported that collembolans able to reproduce sexually, need fertilisation for every reproductive instar. With that regard, Krogh (2008) reported the result of a study aiming at following the oviposition pattern of reproduction. In that study, 24 couples of 25–28 days old, 8th instar, *F. fimetaria* males and females, and 24 single females were isolated and followed for 3 weeks at 20°C. Single females did not produce any eggs and the couples produced 10 and 30 eggs in instars eight and ten, respectively, with a maximum clutch size of 60 eggs. In the same situation, for *F. candida*, 48 and 71 eggs were produced with a maximum clutch of eggs of 114.

Responses of soil organism communities after lindane application were investigated in a Terrestrial Model Ecosystem (TME) study. Collembolans were adversely affected by moderate dosages of lindane in terms of total and species-specific abundance as well as the community endpoints (principal response curves, diversity measures). Recovery was observed within 1 year (Scholz-Starke, 2013).

For acari, the post-embryonic development can take several months. Acari belonging to the Mesostigmata group have only two immature stages before moulting to the adult form. The other three non-parasitic orders (Prostigmata, Astigmata and Cryptostigmata) have six different developmental stages. Inactive forms are very common in acarine population. For example, Cryptostigmata can spend 30% or their annual cycle in moulting or resting stages. Most Cryptostigmata have one generation per year, although larger species or those living in boreal and arctic environments can take 2–3 years to complete their life-cycle. Reproduction is generally bisexual, although some species can reproduce via parthenogenesis. Cryptostigmata females may produce one to six eggs on average which hatch one to 6 weeks later. For Prostigmata, the number of eggs can vary from 10 to 100 depending on the species.

Oribatid mites are usually reported as having long life cycles, extended development, adult longevity, and iteroparity. The time for completion of an oribatid mite's life cycle is dependent on temperature, moisture and the availability of food, and can vary from 5 months to 2 years. In general, small oribatid mites in a warm climate will take less time to complete a life cycle. In field studies conducted in temperate climates, most oribatid mites have shown a generation time of 1 or 2 years (Jordan, 2001).

Acari of the species *Hypoaspis aculeifer* (Acari: Mesostigmata), the standard test species, become sexually mature after 16 days (females) and 18 days (males). A life span between 48 and 100 days at 25°C is reported (OECD, 226).

Microfauna

In several studies, pesticides contributed to the declining diversity and complexity of nematode communities as reported by different specific indices (structural index (SI) and enrichment index (EI)). Moreover, specific nematode genera were indicated as sentinels for recovery and describing the impact of soil management or land-use change. *Mesorhabditis* spp. was a consistent indicator of nutrient enrichment (Zhao and Neher, 2013; Malherbe and Marais, 2015). The resilience of *Cephalobus* spp. to tillage and other agricultural practices was enhanced (Fiscus and Neher, 2002) and *Helicotylenchus* spp. were identified as a candidate soil-health indicator in the tomato agroecosystem studied (Malherbe and Marais, 2015). In general, species of larger body size, such as the longer living, K-selected predaceous nematodes that are somewhat slower moving, would require more time to recover from stress, e.g. PPPs exposure and a larger water film around soil particles (which could also depend on the PPPs applied) to maintain their activity, compared to nematodes in other trophic groups, such as smaller sized, faster moving bacterial feeders with r-selected life strategies (Yeates et al., 2002). Plant parasitic nematodes are rather reactive but can be either target or non-target, depending on the PPP applied. Indirect measures of the resilience and natural attenuation of nematode communities are different traits of their ecological succession, including the fungivore to bacterivore ratio, maturity, and other ecological indices (Ferris et al., 2001).

Timper et al. (2012) found out that nematicides reduced numbers of all trophic groups compared to the control; for bacterial and plant feeders, there was also a consistent, lingering effect of the nematicides the following year at prefumigation. Interestingly, omnivores and predators were not severely impacted by the nematicide treatment; populations of both groups repeatedly recovered by

the following spring from the yearly application of nematicides, with the exception of predators in some cases. The authors highlighted also that the nematicides may have altered the soil community to allow a fungal, bacterial, or invertebrate antagonist of nematodes to increase in abundance, leading to an increase in suppressive service. In addition, although *Caenorhabditis elegans* is a bacterivorous nematode that exhibits exceptional resilience to adverse environmental conditions and different stress, protocols are now available to quantify its resistance to a variety of biotic and abiotic stressors (Keith et al., 2014). This could be a potential tool to estimate the potential recovery and consequent natural attenuation done by bacterivorous nematodes.

Soil microorganisms

Due to their specific traits and short generation time, it has been possible to study internal recovery of microbial populations or communities after exposure to PPPs relatively often. It has been demonstrated and reported (Puglisi, 2012) that microorganisms are often able to recover quite fast from toxic effects after exposure to pesticides. Those effects can be both at the structural and functional levels of the microbial community, as demonstrated by the heterogeneity in the measured and reported endpoints: abundance (number of cells or spores) and biomass (often recalculated from respiration measurements), physiological parameters (e.g. CO2 evolution, net nitrification or mineralisation), measurements of enzyme activities, differences in the structure (PCR-DGGE, PLFA, etc.).

Recovery after pesticide application was reported as occurring from 28 days after application (effects on dehydrogenase) to 114 days (effects on colony forming unit for fungi) (see Appendix C). Some studies have also reported an adaptive response of soil bacteria as shown by the faster recovery of enzymatic activity after repeated applications of a pesticide (Yu et al., 2006; Imfeld and Vuilleumier, 2012). This could be explained by an enhanced mineralisation capacity acquired by the soil microbial community, and by other adaptive changes allowing the microbes to cope with the pesticide.

For mycorrhizae, little information is reported about their potential for internal recovery. Abd-Alla et al. (2000) investigated the effects of the pesticides pyrazophos (fungicide), bromoxynil (herbicide), paraquat (herbicide) and profenofos (insecticide) on arbuscular mycorrhizal (AM) spore number and root colonisation of the legumes cowpea (*Vigna sinensis* L.), common bean (*Phaseolus vulgaris* L.) and lupin (*Lupinus albus* L.). In the case of cowpea plants and common bean, the proportion of root length colonised by AM fungi was significantly decreased with all pesticides used 20 days after planting, but recovery from effects after the application of pyrazophos and bromoxynil was demonstrated after 60 and 40 days, respectively. However, root colonisation of lupin with AM fungi was significantly reduced with all pesticides. The number of AM spores sieved from the rhizosphere of cowpea was significantly decreased with all pesticides after 20 days, but the effect of paraquat had disappeared after 40 days. Except for pyrazophos after 20 days, all the other pesticides significantly reduced the number of AM spores collected from the rhizosphere of common bean after all experimental periods. AM spore formation in the rhizosphere of lupin was inhibited with all pesticides and after all experimental periods.

3.2.2. Potential for dispersal

Macrofauna

Dispersal of earthworms can be categorised as passive through anthropogenic or natural processes and active over the soil surface or through the soil. Both cocoons and adults can be dispersed passively by surface run-off, water currents, heavy rainfall, temporary inundation of a certain area, transported by other animals, e.g. birds or through plant materials and adhesion to soil particles. The active dispersal of earthworms can be triggered by various factors, such as increased earthworm density, low quality habitat or adverse conditions, like heavy rain or flooding, surface applications of irritating fluids, contamination with heavy metals or pesticides, in general, and copper compounds in particular, acid or highly alkaline soils or occurrence of roads and cabins. This was confirmed by Mathieu et al. (2010) who showed in a mesocosm study that dispersal can be reduced by: 1) high habitat quality including the presence of litter; 2) low density; and 3) pre-use of the soil by conspecific individuals that are no longer present.

Data on dispersal rates of earthworms through soil are reported in Table 3, showing mean horizontal movements ranging between 2.5 and 14 metres per year (m/y) (Eijsackers, 2011; Emmerling and Strunk, 2012; Dupont et al., 2015). For *A. caliginosa*, maximum dispersal of 72 m in 8 years was reported. In the case of *L. rubellus*, a dispersal between 5 and 11 m/y has been measured. Overall, in agricultural sites, limited variation has been reported in the dispersal rate between different species, and earthworm-population development started after an adaptation period in the range of 2–6 years after introduction.

All types of earthworm species show the ability to disperse over the soil surface by crawling at night. For example, *L. terrestris* has been shown to crawl 19 m in one night and *A. longa* 23 m. No directionality in crawling has been demonstrated. However, earthworms can detect and avoid adverse conditions as reported above and, thus, colonisation by earthworms may not occur for years in the case of soil contaminated by persistent substances (Eijsackers, 2011).

Caro et al. (2013b) recorded a high variability within each earthworm functional group concerning dispersal behaviours. Habitat quality significantly influences the dispersal rates of both anecic and endogeic species. In a homogeneous environment, anecics dispersed further and in greater proportion than the majority of endogeics. Overall, the authors concluded that anecic species might show more active dispersal than most endogeic ones.

Earthworms dispersal behaviour can be triggered by environmental conditions, such as habitat quality. In this respect, Mathieu et al. (2010) reported that 90% of individuals belonging to the endogeic species *Aporrectodea icterica* dispersed when inoculated into a low quality soil, while only 20% dispersed when inoculated into a soil which was demonstrated as largely preferred by earthworms (see Figure 2).

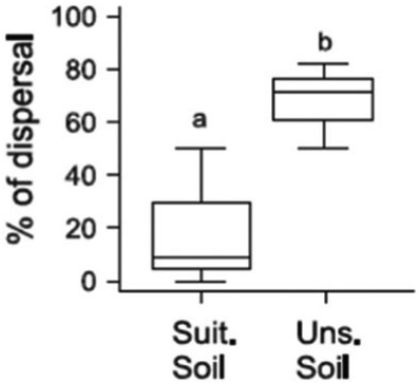

Figure 2: *Aporrectodea icterica* dispersal rates in response to soil properties. Suit: suitable soil (high pH, high org. matter); Uns: unsuitable soil (sandy soil, low pH). Reprinted from Mathieu et al. (2010), Copyright (2010) with permission from Elsevier

Table 3: Mean dispersal rate of earthworms species in various habitats (from Eijsackers (2011) and Emmerling and Strunk (2012)

Species	Land use/soil/environment	Dispersal rate (m/y)
Lumbricus rubellus	Grazed grassland	7–8
	Arable land	14
	Peat soil	> 10
	Arable polder	14
Aporrectodea caliginosa	Grass strips orchards	6
	Grazed grassland	9–11
	Arable land	7
	Grassland	6
	Irrigated desert soil	3.5–5
	Pasture	10
	Grassland/reclaimed peat	2.5–10
	Arable polder soil	7
Allolobophora chlorotica	Grass strips orchards	4
Aporrectodea longa	Grazed grassland	5–8
	Grassland	6
Lumbricus terrestris	Grazed grassland	4
	Grassland	1.5
Octolasion cyaneum	Grassland and arable soils	8

The greater part of terrestrial gastropod diversity comprises very small animals living as detritivores in the litter layer (< 1 cm diameter in greatest dimension), some even have maximum diameters of < 1 mm (see e.g. Barker, 2001; Sturm et al., 2006). They occur in all kinds of agricultural habitats (as grassland, acres, specialised crops, seminatural habitats) and have often rather specific preferences in terms of habitat and environmental conditions (see e.g. Kerney et al., 1983).

Barker (2001) states that the dispersal abilities of terrestrial gastropods are so low that it can be assumed that mating will be predominantly driven by inbreeding at the level of the local population. Their ability to recolonise disturbed areas is low and affected by various environmental factors, such as the height of the corn (Wolters and Ekschmitt, 1997). For rape fields, the same authors reported that several snails only invaded about 3 m into fields from woodlands and hedges. Hof and Bright (2010) found that the number of terrestrial gastropods significantly decreased with increasing distance from the field edge of arable fields.

Mesofauna

Dispersal is an important characteristic of mesofauna with implications for impacts and recovery of PPPs. Kattwinkel et al. (2012, 2015) reviewed the literature on recovery and concluded that Collembolan species reacted significantly differently to population perturbation (including the recovery pattern), meaning that a coarse taxonomic assessment might not be sufficient to detect adequately effects of pesticides. For example, when changing the same plots from the treated to untreated management, responses of individual species varied, e.g. numbers of the species *Entomobrya nicoleti* remained close to zero, whereas the abundance of *Isotoma viridis* were the highest recorded during the study. They also concluded that unexposed field margins play a key role as source of recolonisation confirming the role of buffer zones for recovery for mobile surface dwelling collembolan.

There is a considerable body of evidence for the importance of dispersal. Rantalainen et al. (2005) reported the ability of various members of the detrital food web to colonise newly established habitat patches under field conditions, showing that the presence of habitat corridors promoted community diversity. However, rates of movement, although varied are generally low. Dispersal rates for the fungivore species of Collembola, *Onychiurus armatus*, using connected distinct patches of two different soil types covering a distance of 40 m, ranged from 0.020 to 1.42 per day suggesting that on average species moved less than 10 centimetres per day (cm/d). Dispersal depended on population density, soil type and length of fungal mycelium, being inversely proportional to mycelial length, especially in a sandy soil. When a soil patch at 40 cm distance from the release point was enriched with a favoured food item, dispersal rate was increased by more than four times (Bengtsson et al., 1994). The role of hedgerows for external recovery (recolonisation) of springtails has also been investigated and demonstrated, especially in arable fields. Habitat preference and dispersal ability of different collembolan species have also been investigated by Auclerc et al. (2009) at a small scale study conducted in France. The authors showed that 6% of the identified species were land-use generalists (not restricted to a given habitat), 30% were soil generalists and 36% recolonised defaunated soil blocks within a week. The results also demonstrated discrepancies between preference in land-use and soil, indicating that land-use specialists may not always be also soil specialists. However, food availability was suggested as stimulating dispersal considering that the meadow soil was more attractive than the forest, whatever the land use preference of the species. In addition, it was also shown that dispersal ability might not be predicted based on the morphological features (antenna, legs, etc.) of the species.

Other factors that can alter dispersal rates include reproductive strategy and pheromones. There is an indication that parthogenetic species may colonise more quickly (Chahartaghi et al., 2009). Recovery and recolonisation of Collembola may be also enhanced by the existence of pheromones which induce aggregation. As mating in Collembola may be indirect, involving deposition of spermatophores by males and subsequent taking up by females, aggregation may increase the efficiency of reproduction (Verhoef and Nagelkerke, 1977; Verhoef, 1984).

Since dispersal is important but limited there are implications for the interpretation of field study data. According to Duffield and Aebischer (1994), the recovery of invertebrate population also depended on the size of the treated plot. In addition, two different recovery patterns were identified: (i) recovery progressing from the edge to the centre of treated areas; (ii) more rapid recovery in the centre of the large treated areas. The first recovery pattern was mostly associated with the predatory groups such as Carabidae, Staphylinidae and Linyphiidae and it can be associated with a recolonisation of the pesticide-treated plots from the untreated surroundings. The second recovery pattern was associated with the prey groups such as Aphididae and Collembola. The recovery appeared to be

faster in areas with less predation pressure. The results suggested, as also reported by Kattwinkel et al. (2012, 2015), that recovery assessment in small in-field areas might be a source of uncertainty since it could underestimate the pesticide effects on large predators, while overestimating them on microarthropods. This was extensively addresses by the EFSA PPR Panel (2015a) when dealing with the risk of intended uses of PPPs to non-target arthropods. Some patterns might be also valid for the larger Collembola species living on the soil surface and able to move significant distance between fields and edge-of-fields. One of the major threats to biodiversity is landscape fragmentation as it can result in the transformation of continuous (hence large) habitat patches into isolated (hence smaller) patches, embedded in a matrix of another habitat type. In turn, this leads to a loss of biodiversity, especially if species have poor dispersal abilities, such as Collembola (Martins da Silva et al., 2012). A recently created habitat might suffer from a reduced biodiversity because of the absence of adapted species that need a certain amount of time to colonise the new patch (e.g. direct metapopulation effect). Thus, landscape dynamics leads to complex habitat, spatiotemporally structured, in which each patch is more or less continuous in space and time. Patches can also display reduced biodiversity because their spatial or temporal structures are correlated with habitat quality (e.g. indirect effects). Heiniger et al. (2014) demonstrated that habitat temporal structure is a key factor shaping collembolan diversity, while direction and amplitude of its effect depend on land use type and spatial isolation.

Soil microorganisms

For a long time, the 'Baas-Becking hypothesis', stating that 'everything is everywhere, the environment selects' (Beijerinck, 1913; Baas-Becking, 1934; Fierer, 2008), made a strong imprint on thoughts and views regarding microbial biogeography. It is well known that microbial communities can exhibit spatial variability at scales ranging from millimetres to thousands of kilometres and that microbial community composition in natural environments can be influenced by a large number of biotic and abiotic environmental factors. However, the impact of specific aspects of the environment on the spatial patterns of microorganisms is still poorly understood. Typical features of microbial communities, such as large population sizes and short generation times, may result in biogeographical patterns. However, unlimited microbial dispersal may lead to constant turnover and increase in genes flow (Eisenlord et al., 2012). For example, many phylogeographical and population genetic studies on plant pathogenic fungi, but also on wood decay species, have reported efficient dispersal and gene flow at a regional or even continental scale. According to Finlay (2002), free-living microbial eukaryotes are probably sufficiently abundant to have worldwide distribution. Accordingly, prokaryotes, which are much smaller and several orders of magnitude more abundant, are even less likely than microbial eukaryotes to be restricted by geographical barriers.

In addition, in the case of fungal spores, for example, dispersal patterns can affect gene flow, population structure and community structure. Dispersal mode can vary among different fungi. While epigeous fungi are mainly transported by wind, the majority of hypogeous fungi are biotically dispersed since they have fewer opportunities than epigeous fungi for being passively dispersed. For example, fungivorous mammals and invertebrates may be an important dispersal agent for many ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) fungi that form sporocarps. It has been reported that arbuscular mycorrhizal fungal spores can remain viable after passing through digestive tracts of earthworms, sowbugs, and crickets. Besides ingestion, dispersal by adhesion to external surfaces of in-soil organisms is another mode of dispersal for spores of soil fungi (Lilleskov and Bruns, 2005).

4. Steps to derive specific protection goal options

Regulation (EC) No 1107/2009 defines general protection goals that aim at protecting biodiversity and ecosystems. It is thus necessary to define specific protection goals (SPGs) with the scope of implementing this general protection into explicit and viable mandates for risk assessors who need to know what to protect, where to protect it and over what time period. A procedure to define specific protection goals was developed by EFSA in consultation with stakeholders (EFSA PPR Panel, 2010a). Final decisions on the choice of specific protection goals need to be made in consultation with risk managers. In the PPR Panel Opinion (EFSA PPR Panel, 2010a), several steps are proposed in order to identify and to justify specific protection goals for aquatic and terrestrial organisms that may be affected as non-target organisms by the use of PPPs.

The role of EFSA's risk assessment is, therefore, to propose possible SPG options based on environmental and ecological criteria (and related exposure-assessment goals), acknowledging existing general protection goals described in the relevant EU Regulation or Directive and regulatory data

requirements. These SPG options, as well as a description of the possible environmental consequences of each option, should be proposed and discussed with the risk managers. The role of risk managers is to select SPG options, or to amend SPGs proposed by risk assessors, that should form the basis of agreed environmental risk assessment (ERA) decision schemes (subsequently included in guidance documents). The choice by risk managers of the DG SANTE and the EU Member States is based on a cost-benefit evaluation, also using economic and political criteria and acknowledging consequences for human well-being (health and economic benefits) as well as environmental costs.

Based on the overarching ecosystem services concept, which was introduced in the so-called Millennium Ecosystem Assessment (MEA, 2005), the PPR Panel identified those ecosystem services that could potentially be directly or indirectly (e.g. via trophic interactions) affected by the normal agricultural use of plant protection products. The groups of in-soil organisms that are key drivers or service providing units (SPUs) for those ecosystem services were then identified. SPG options have to be proposed for each combination of a key driver and ecosystem service.

The first step in the definition of SPGs is the identification of ecosystem services that are considered important and are provided by agricultural ecosystems. By means of describing services that mankind receives from ecosystem performance, the value of abstract ecological entities and processes become more explicit. Several classification schemes for ecosystem services have been proposed, e.g. MEA, 2005; CICES (http://cices.eu/) and TEEB (http://www.teebweb.org/). In this Opinion, in accordance with other Opinions and Guidance of EFSA on the topic (EFSA PPR Panel, 2010a; EFSA Scientific Committee, 2016b), a list of ecosystem services based on the MEA source has been used since it is widely recognised and adopted. The Millennium Ecosystem Assessment (MEA, 2005) noted, however, that 'modifications of ecosystems to enhance one service generally have come at a cost to other services due to trade-offs'. The impacts of these trade-offs should be clearly described also for ecosystem services in agricultural landscapes, so that risk managers can decide whether and to what extent costs of trade-offs should be tolerated. In this respect, MEA (2005) claims that 'many of the costs of changes in biodiversity have historically not been factored into decision-making'.

Seven ecosystem services were identified as being driven by in-soil organisms in the agricultural landscape. These services are:

- Genetic resources, biodiversity. In-soil organisms are extremely diverse and contribute highly to the biodiversity of agricultural landscapes.
- Education and inspiration, aesthetic values and cultural diversity. In-soil organisms support with their activity the formation of typical structures in agricultural landscapes, delivering aesthetic values, cultural heritage and sense of place. The aesthetic value of soils is widely acknowledged.
- Nutrient cycling. The cycling of nutrients in soils is the basis for terrestrial life. Dead organic matter from above- and below-ground is degraded by detritivores and finally mineralised by microorganisms. Mineralised nutrients can be then taken up by plants.
- Regulation of pest populations and of disease outbreaks. In-soil organisms are valuable antagonists of soil-borne pests affecting crop-plant species and have the potential to control the outbreaks of plant diseases.
- Soil remediation, natural attenuation. In-soil organisms degrade a variety of compounds in soils and contribute to the natural attenuation of xenobiotic soil pollution, including pesticides and their residues.
- Soil-structure formation, water retention and regulation. In-soil organisms are important drivers of soil-structure formation and maintenance. The activity of soil organisms modulates aggregate formation, alleviate soil compaction and regulate soil water-holding capacity.
- Food provision, food-web support. In-soil organisms are part of the below-ground food web and are the link to above-ground consumers. They are providers of secondary production and support biodiversity at a higher trophic level.

The second step in the definition of SPGs **is the characterisation of the main drivers** behind the ecosystem services deemed to be important in agricultural landscape. In the chapters dealing with the respective SPGs in the present Opinion, in-soil organisms' species and/or groups have been identified as having, through their activity or presence, major influences on the service to be preserved. In the Guidance of the Scientific Committee (EFSA Scientific Committee, 2016b), the definition of 'key driver' applies to 'service providing unit'. *SPUs are defined as the structural and functional components of ecosystems necessary to deliver a given ecosystem service at the level required by service beneficiaries* (adapted from Luck et al., 2003; Vanderwalle et al., 2008).

The third step is the determination of the drivers' ecological entity to be considered with respect to the ecosystem service assessed. The PPR Panel (EFSA PPR Panel, 2010a) suggested to differentiate between the ecological entities 'individual', '(meta)population', 'functional group' and 'ecosystem'. The concept is based on the assumption that addressing organisms at one level of organisation will protect those at a higher level of organisation. For example, if the ecological entity to be protected is the 'individual', the entities 'population', 'functional group' and 'ecosystem' will implicitly be protected. The ecological entity addressed in the assessment is identified in the definition of every specific protection goal. In general, non-target organisms other than vertebrates are not protected at an individual level. In the case of SPGs for in-soil organisms, the ecological entities relevant to deliver different ecosystem services are either the populations of species or the functional group (see below).

The fourth step is the determination of the drivers' attribute to be measured in the assessment. Changes in behaviour, on survival and growth, in abundance/biomass, in a process rate or in biodiversity are suggested by the PPR Panel (EFSA PPR Panel, 2010a) as possible measurements to be made for the different drivers considered. In the case of in-soil organisms, and according to the ecological entities considered in the previous step, the most reasonable attribute to measure will be abundance and/or biomass (see details below).

The fifth step is the determination of the magnitude of effect on the drivers that could be tolerated regarding the impact on the respective ecosystem service without affecting the general protection goal. In the following, a partitioning of magnitude of effects is proposed deriving from general effect classes in ecotoxicology. Changes in effects size are described following dose scaling classes. It is noted that these classes describe the magnitude of effects on the drivers attributes and do not aim at assessing the 'adversity' of the observed effects (i.e. 'effect' and not 'risk'). Which of these effect classes are considered 'not adverse' in terms of this Opinion is described in the SPGs for every driver/SPU (see Section 6.2). From these effect classes, the pertinent one is chosen for final SPG Option proposal, depending on the organisms' traits that determine, e.g. sensitivity, life cycle or recovery potential.

Scaling of magnitude of effects on population/functional group/biodiversity

- Large effects: pronounced reduction, corresponding to effects above 65%;
- Medium effects: reduction comparable to median effect size (i.e. corresponding to median effect class of 50%; effects between 35% and 65%);
- Small effects: reduction above No Effect Level and below medium effects (above 10% and below 35%);
- Negligible effects: reduction up to No Effect Level (comparable to 10%).

The three options large, medium and small effects resemble the ecological recovery option while the option negligible effects is comparable to the ecological threshold option as defined in the aquatic guidance document (EFSA PPR Panel, 2013). Especially the definition of 'negligible' has been often matter of debate, also on recent Panel publications (e.g. Bakker, 2016). This is possibly due to misunderstandings regarding the addressed target. The Panel refers here to effects on the 'assessment endpoint', namely which magnitude of effect might be tolerable for in-soil organisms as drivers of ecosystem services in order to still meet the proposed SPG options (e.g. Munns et al., 2016). This target has to be distinguished in principle from what will be the 'measurement endpoints' (or 'measure of effects', USEPA, 1998, 2004), which are the measurable characteristics related to the chosen assessment endpoints (Suter, 1993). The term 'negligible' is not used in this Opinion in relationship to exposure of non-target organisms (e.g. Mackay, 1988), nor it relates here to effects that are 'not adverse' (i.e. not 'negligible risk', e.g. Duffus et al., 2007; Barnard, 1990; Boekelheide and Andersen, 2010; Dorato and Engelhardt, 2005; Keller et al., 2012; Ricci et al., 1987). In terms of this Opinion, the *definition of "negligible effects" on ecological entities reads as follows: no increases in the frequency of effects between exposed and unexposed groups*. This definition relates as close as possible to the continuum of effects in a dose–response relationship and does not judge at this point on which effects are acceptable (e.g. Barnard, 1990). By contrast, the SPG options will mark the points at which the effects on the drivers gain such magnitude that they can be considered adverse. For example, EFSA PPR Panel (2015a) describes that the magnitude of effects that can be tolerated on non-target arthropods (NTA) might be clearly above 'negligible' – as long as the NTA abundances are able to recover in a given time frame. Only above this threshold or tipping point, the service provision cannot be guaranteed anymore and the magnitude of effects on the ecological entities becomes clearly adverse.

It should not be a matter of debate that the measurement of negligible effects has to be based in practice on careful biological and statistical analysis. Every measure of effects in experimental or modelling approaches will have characteristic explanatory values and care should be taken not to use underpowered studies to establish no effect levels (e.g. Bross, 1985; Millard and Bross, 1987; Hoekstra and van Ewijk, 1993; Parkhurst, 2001; Dixon and Pechmann, 2005).

Regarding the magnitude of effects on in-soil organisms arising from several years of PPPs exposure in an agricultural context, relevant measurement endpoints are still to be agreed in the scientific community. If the assessment of these effects is based on population models that address effects of PPPs on species, efforts should be made in order to identify those simulation endpoints that can be related to the magnitude of effects in the SPG as defined above. In general for non-target organisms, the endpoint of population size has been used (e.g. Schmitt et al., 2016), but other viable endpoints are population growth rate (e.g. Forbes & Calow, 2002), population viability. These were assessed using a vole population model by Wang & Grimm (2010) who concluded that population size is the most sensitive endpoint, However, distribution as well as abundance is an important characteristic of potential response of non-target organisms (see EFSA PPR Panel 2015a; Topping et al., 2015a).

Depending on the endpoints that will be chosen in future for assessment of PPP effects on population persistence, negligible, small, medium and large effects will have to be defined. Since modelling endpoints integrated several years of PPP application ('system approach', see also Section 7.3) tolerable effects might be of lower magnitude than those defined for community assessment at a local scale (e.g. in- or off-field). On the one hand, year on year decline in abundance should not be observed. On the other hand, negligible effects should also account for population-range restrictions: here, not only individual abundance but also range of occupancy should not be reduced by more than a level that will be considered negligible.

In terms of this Opinion, the definition of possible acceptable magnitude of effects as percentage reduction compared to a 'control' applies to a defined context. For example, in an agricultural system supporting a high diversity of in-soil organisms, a given reduction (e.g. 50%) may still retain the function represented by the SPG. In contrast, in landscapes with very low in-soil diversity, the acceptability of effects might be at a far lower magnitude level, e.g. removing 50% of two species may be critical. This context dependency applies to all proposed SPG options for in-soil organisms. Please refer to Section 7.3 for the concept of defining baselines for risk assessment in multiple contexts. For services supported and provided by in-soil organisms, it difficult to define effect thresholds marking tipping points for ecosystem functioning and the provision of the service of interest. This is due to the lack of knowledge on the detailed quantitative relationships between species and functions in soils. If no absolute threshold can be defined, maximum magnitudes of effects on drivers/SPUs are suggested marking the acceptable limits, in scientific terms, for the maintenance of the assessed service at a desired rate and ultimately for the general protection goal (EFSA PPR Panel, 2010a). This means that, if such limits are breached, severe consequences for the ecosystem functioning and for stakeholders who rely on certain services can be expected. These 'limits of operation' mark the upper range of the magnitude of effects in the different SPG options. The lower end of magnitude of effects in the SPG options is set where no or negligible effects are observed on in-soil drivers, with no or negligible impact on the provision of the specific ecosystem service.

For in-field as well as off-field areas, the tolerable magnitude of effects should take multiple PPP applications according to typical PPP 'spray schedules[10]' into account. This could suggest a lower level of tolerable effects for single PPP applications, especially in-field, if the intended use fits in an application scheme that includes several other PPPs with potential effects on in-soil organisms in the crop. Multiple applications of several PPPs in typical schedules should also be taken into account when addressing the recovery of in-soil organisms (please refer to Section 7.4). This is currently not supported by the regulatory framework for approval of active substances/authorisation of PPPs, however, the Panel would strongly recommend that this aspect should be taken into consideration when setting SPGs.

The sixth step is the determination of the temporal scale to be considered together with the magnitude of tolerable effects. This step is of particular importance when addressing effects other than negligible, since it implies that some effects might be tolerable as long as ecological recovery occurs within a specified period. As stated in the EFSA Guidance on the risk assessment for aquatic organisms (EFSA PPR Panel, 2013), when including 'recovery to identify (un)acceptable effects, all

[10] Overall pesticide input and application patterns on a field.

relevant processes that determine population viability and the propagation of effects to the community-, ecosystem- and landscape-level are to be considered'. In this respect, multiple applications of PPPs might pose a constraint to recovery processes in agricultural landscapes – in particular the consecutive PPP uses throughout crop-spraying schedules.

Considering the ecosystem services identified above in Step 1, their timely provision might be of central importance. In-soil organisms may display uni-, semi- or multivoltine life-history strategies (e.g. Lavelle and Spain, 2005). For univoltine and semivoltine species, full recovery from chronic effects might only be observed 1 year or more after PPP use. Therefore, the Panel considers time lapses of 1 year or more as relevant for the demonstration of, e.g. long-term effects on in-soil species that may emerge after several year of PPP use or for the demonstration of recovery of species with a long life cycle. Therefore, the temporal scale of SPGs as assessment endpoints diverges from the time scale of measurement endpoints, which should cover also the life cycles of vulnerable species (see Section 7.9).

Regarding the ecosystem services driven by in-soil and having an impact on other organisms (e.g. 'pest control' or 'food web support'), time ranges for full recovery that are greater than the growing season are most likely not adequate to satisfy the protection goals. The temporal scaling of effects on in-soil organisms drivers may be classified as follows:

- 6 months: not considered adequate to satisfy protection goals unless effects are negligible. Negligible effects are considered as no effect level;
- Months: maximum of 6 months;
- Weeks: up to 4 weeks;
- Days: up to 7 days.

The seventh step is the determination of the spatial scale. Please, refer to Section 6.1 for the definition and the choice of spatial scale in the risk assessment of in-soil organisms.

5. In-soil organisms and ecosystem services in agricultural landscapes

5.1. In-soil organisms as drivers of the provision of genetic resources, biodiversity

The Introduction to this Opinion noted that the overall protection goal regarding the environment according to good agricultural practice is that PPP 'shall have no unacceptable effects on the environment, having particular attention to [...] its impact on non-target species, including on the ongoing behaviour of those species; [and] its impact on biodiversity and the ecosystem' (Regulation (EC) No 1107/2009 on plant protection products).

The EFSA Scientific Committee gives guidance on how to set accurately (specific) protection goals that should cover the general protection goal 'biodiversity' laid down in the legislation of several regulated products using the framework of the 'ecosystem service approach' (EFSA Scientific Committee, 2016b). The ecosystem service approach has been introduced for environmental risk assessment by EFSA in 2010 (EFSA PPR Panel, 2010a) and has been successfully applied since then in several scientific outputs (e.g. EFSA PPR Panel, 2013, 2014a, 2015a,b).

Biodiversity can be defined as the 'variety of life, including variation among genes, species and functional traits'(Cardinale, 2012). Several aspects of biodiversity can be assessed when it comes to 'measuring' the diversity of organisms in a specified system. For example, richness 'is a measure of the number of unique life forms', while evenness 'is a measure of the equitability among life forms', and heterogeneity 'is the dissimilarity among life forms' (Cardinale, 2012).

Some general aspects on the importance of wider biodiversity that are agreed upon in the scientific community and are relevant for ecosystem services in agricultural landscapes are as follows:

- There is unequivocal evidence that biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, and decompose and recycle biologically essential nutrients.
- There is strong evidence that biodiversity provides stability of ecosystem functions over time.
- The impact of biodiversity on any single ecosystem process is non-linear and saturating, such that a change in process rates accelerates as biodiversity loss increases.
- Diverse communities are more productive because they contain key species that have a large influence on productivity, and differences in functional traits among organisms increase total

resource capture. Loss of diversity across trophic levels has the potential to influence ecosystem functions even more strongly than diversity loss within trophic levels.
- Functional traits of organisms have large impacts on the magnitude of ecosystem functions, which give rise to a wide range of plausible impacts of extinction on ecosystem function (Cardinale, 2012).

The wide definition and some peculiarities of biodiversity measurements, however, make the assessment of this general protection goal challenging.

First, as reviewed by the PPR Panel (EFSA PPR Panel, 2015a), 'normal operating ranges' of biodiversity differ between different ecosystems. As a general rule, no single biodiversity value – whatever measurement endpoint is chosen – can be defined as being appropriate for all ecosystems. In fact, apart from some ecosystems claimed to be 'highly diverse', an increase in species diversity owing to the additional presence of generalists on top of specialists might be an indication of the onset of disturbance (e.g. Begon et al., 2006). In the framework of this Opinion, extensively managed organic farmed fields with comparatively low input of PPPs and high biodiversity could act as a reference system to derive appropriate 'normal operating ranges', especially for in-field areas in agricultural landscapes (e.g. Moreby et al., 1994; Hole et al., 2005; Tuomisto et al., 2012; literature review in Brühl et al., 2013; Rutgers et al., 2008).

Second, the evaluation of biodiversity relates to comparisons between areas or between time points, and different biodiversity measurement endpoints might apply and deliver different metrics (e.g. so-called alpha-diversity at local scale, beta-diversity between different fields or gamma-diversity at landscape scale). No single measurement endpoint of biodiversity can be proposed as appropriate for all organisms because several ecosystem services driven by non-target organisms are important at the same time in agricultural landscapes and different spatial assessment scales might be relevant for different ecosystem services. The spatial scales defined for the assessment of potential effects on in-soil organisms after exposure to PPPs should be related to the traits of these organisms regarding their dispersal and recovery capacities. It is anticipated that assessment of local aspects of diversity of in-soil organisms in the in- and off-field areas might deliver the appropriate metrics (see Section 6.1).

As defined in the chapter above, the initial step to derive SPGs is the identification of relevant ecosystem services (ES) and of the so-called key drivers or service providing units (SPUs). The SPUs – as defined by EFSA Scientific Committee (2016b) and employed in the present Opinion – are 'the structural and functional components of biodiversity necessary to deliver a given ecosystem service at the level required by service beneficiaries' (see also e.g. Vanderwalle et al., 2008).

EFSA Scientific Committee (2016b) points to the fact that several types and categories of ES (e.g. ES underpinning plant/animal production or other services relevant to society) have elements that either depend on or are influenced by biodiversity. In particular, the provisioning service 'genetic resources' can be also suitably defined in order to address components of the general protection goal 'no unacceptable effects on non-target organisms, biodiversity and the ecosystem' as defined in the Regulation 1107/2009.

The relevant SPUs are those components of biodiversity that are 'necessary' for in-soil organism communities in the context of agricultural landscapes (EFSA Scientific Committee, 2016b). For this purpose, it was concluded that in-soil organism SPUs regarding the ecosystem service 'biodiversity and genetic resources' should address the following specific aspects:

1) the biodiversity of in-soil organisms as a general protection goal, i.e. the 'intrinsic' value of biodiversity as regulated good;
2) the reliability of the performance level of other SPUs among in-soil organisms, with particular reference to changing environment and multiple stress, i.e. the supporting service of biodiversity for other ESs; and
3) an option value for biodiversity and genetic resources, i.e. the provisioning service of diversity in order to take advantage of ecosystem services also in the future.

Microorganisms pose particular challenges when it comes to defining diversity in terms of the entity 'species'. Soil fauna and soil microorganisms will therefore be dealt with in separate sections.

Soil fauna

The first aspect of the ecosystem service 'biodiversity and genetic resources' addresses the *diversity level needed to support communities of in-soil organisms* in agricultural landscapes. In the discussion about definition of biodiversity as a general protection goal, EFSA (2014a) states that the species

diversity *per se* is often defined as 'structural biodiversity' versus a so-called 'functional biodiversity'. Structural biodiversity delivers through the magnitude of the different species' traits the 'functional biodiversity'. A functionally diverse system is also likely to host a higher structural diversity than one with low functional diversity. However, functional biodiversity focuses on the specific function that (a group of) species exerts in the processes of interest.

A clear assignment of a species to a functional group is, however, not possible. A single species has several functions, as several traits can be assigned to every species, and every species in turn is uniquely characterised by its trait configuration (e.g. Gardner et al., 2014). Given that the knowledge on the functions of the extremely diverse soil fauna in ecosystem processes is far from being comprehensive, it is not recommended to address 'biodiversity' at a merely functional level. This helps in defining SPGs that might otherwise being considered vague and prone to be contested (Arsel and Buscher, 2012; Büscher et al., 2014; Schroter et al., 2014; Kull et al., 2015).

It is accepted that the diversity of species stands behind processes that might eventually result in desirable services (see above), but the species composition over time in one functional group might change drastically at the expense of the level of diversity. In an extreme scenario of species erosion, one function of interest may be performed by a single species, with far reaching consequences not only for the goal of protecting biodiversity, but also for the stability and resilience of the system.

Functional groups of in-soil organisms are hierarchically organised in soils (functional domains *sensu* Lavelle, 2002; Anderson, 1988). Species assigned to different functional domains interact with each other, especially at the lower trophic level. Interactions between the component of the soil food web, are of biological, biophysical and biochemical nature (Brussaard et al., 2007b and references therein). Their overwhelming complexity indicates that species shifts in one functional group might interfere with the structural diversity of other functional groups (e.g. Sheenan et al., 2007). The most difficult species' benefits to assess are those occurring via trophic cascades and the so-called 'consumer connections', since they might be wholly unanticipated or build up unexpectedly, with far reaching consequences even in species-rich communities (Duffy, 2002; Mouillot et al., 2013; Gascon et al., 2015).

Therefore, the SPUs for soil fauna addressing the level of genetic resources needed to support biodiversity of in-soil organisms as a general protection goal are the different species of soil invertebrates belonging to the micro-, meso- and macrofauna. Populations of different species are the relevant entities of these key drivers, independently of whether species belong to the same functional group or not (e.g. several earthworm species).

The second aspect that has been identified above, as relevant, to the ecosystem service 'biodiversity and genetic resources' and driven by in-soil organisms is the *impact of diversity on the reliability of the ecosystem functional responses* (stability and resilience) in a changing environment and under the impact of multiple stressors. As stated by Naeem and Li (1997), 'reliability refers to the probability that a system will provide a consistent level of performance over a given unit of time'. Here, we do not refer to the general relationship 'higher diversity, better ecosystem performance', but to the fact that agricultural fields (and often also their surrounding habitats) are hot spots of drastic environmental changes and repeated PPPs applications. Since a 'system that is functioning properly is one that will persist despite natural environmental fluctuations' (Palmer et al., 1997), we argue that the timely and simultaneous performance of different services requires here a different level of diversity than in systems with more constant environmental conditions.

There is a continuum of hypotheses linking soil species diversity to a better functional performance of ecosystems, with two opposite far ends. One is the 'rivet' hypothesis, which suggests that each species has a unique effect on ecosystem function, and the opposite is the 'redundant species' hypothesis, which suggests that only a minimum number of species is necessary for ecosystem function. Even if some studies seem to support the hypothesis that species are redundant and saturation of functions is reached at low level of species richness, Wolters (2001) argues that the potential effect of associated diversity implies that 'redundant species may gain functional significance by interacting with functionally important species'. These interactions between redundant species and keystone species, the loss of which might seem more critical in the first term (Gitay et al., 1996), are best explained in Figure 3 below.

Elimination of redundant species may affect the steepness of the relationship between species diversity and function. Feedback effects of functionally important species may accelerate this process; the same is true for disrupted facilitative or mutualistic interactions (Wall and Moore, 1999; Zhang and Zhang, 2015; Mod et al., 2015). The complexity of interaction between species makes the determination of the 'number' of species that a system can afford to lose extremely difficult. Some species loss can be compensated for, but, if the erosion process continues, a 'tipping point' for

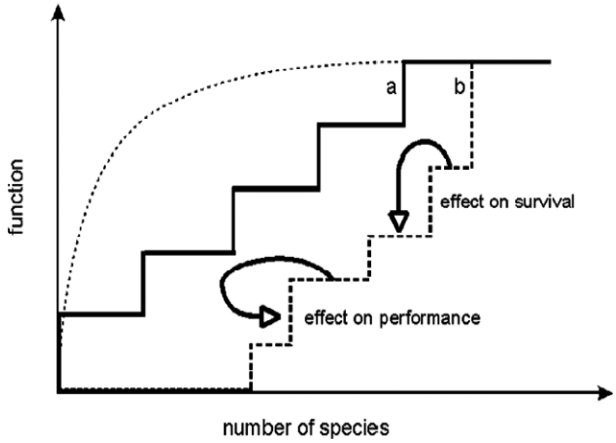

Figure 3: Average functional effect of the elimination of species from a soil biota community composed of a very rich group of redundant species and a small group of functionally important species. Graph a) no interaction between species; Graph b) effect of redundant species on survival and/or performance of functionally important species. From Wolters (2001) copyright Elsevier

ecosystem functioning and ecosystem service provisioning is reached and the system may slip in a different status or definitively collapse (e.g. Lever et al., 2014). Therefore, the concept of species redundancy may be regarded as a 'redundant concept' (Gitay et al., 1996), above all in the case of heavily disturbed ecosystems as agricultural fields. Here, species redundancy is a valuable commodity, since each species tolerates only a limited range of climatic and biotic conditions and any change in environment beyond these conditions leads to poor performance or to extinction. As Brussaard et al. (2007a,b) pointed out, if there are several species in a functional group, some species are likely to survive any extreme event, ensuring continuation of the services provided (e.g. 'insurance hypothesis' Yachi and Loreau (1999); Naeem (1998)). The species diversity within a functional group expands the range of conditions over which ecosystem services are performed (Ferris and Tuomisto, 2015). Agricultural soils undergo drastic changes compared with natural systems and soil communities do experience a range of physical and chemical management stressors. Therefore, the SPUs for in-soil organisms addressing the supporting service of diversity for the reliability of the ecosystem functional responses under the impact of multiple stressors in agricultural areas are again the different species of soil invertebrates belonging to the micro-, meso- and macrofauna. The relevant entities of these key drivers are the populations of different species, independently of whether species belong to the same functional group or not. Surely, also phenotypic erosion will have adverse effects on species diversity. In changing environments under high disturbance, high phenotypic variance cannot be sustained *ad infinitum* without external input (Norberg et al., 2001). This has to be considered when defining the magnitude of tolerable effects and the temporal scale of specific protection goal options for in-soil organisms.

The third aspect that we consider when addressing the ecosystem service 'biodiversity and genetic resources' of in-soil organisms in agricultural fields is the identification of SPUs to *deliver diversity and genetic resources as an option value*, i.e. a provisioning service in order to take advantage of ecosystem services also in the future.

Several studies have detected diverging effects of species with apparently very similar traits on measurable ecosystem processes at different levels of resolution (e.g. Cragg and Bardgett, 2001; Pieper and Weigmann, 2008). What is more, the unique importance of rare species to ecosystem function has been demonstrated also in species-rich ecosystems, where high functional redundancy is likely and should buffer the system against species loss (Mouillot et al., 2013; Gascon et al., 2015). Acknowledging these relationships for services that are currently known and measurable and top rated as valuable to mankind, means that similar diversity levels should be granted in order to preserve the option to use these or other services in the future. The *option value* should allow also in future the use of services like genetic resources that are provided by biodiversity. These services might be already known at present – but their attributed value change in future; or these are services that have yet to be quantified.

Summarising the arguments on the three key aspects above, 'maintaining multiple ecosystem processes at multiple places and times requires higher levels of biodiversity than a single process at a single place and time' (Cardinale, 2012). The Panel identifies the SPUs for 'biodiversity and genetic resources' of in-soil organisms as the different species of in-soil invertebrates belonging to the micro-, meso-, and macrofauna. Populations of different species are the relevant entities of in-soil key drivers, independently of whether species belong to the same functional group or not. This is in agreement with the guidance of the EFSA Scientific Committee (EFSA Scientific Committee, 2016b) that states the following: 'when the aim is to maintain specific populations and biodiversity, structural endpoints need to be selected as the ecological entity' to be protected.

Soil microorganisms

Collectively, microorganisms represent more of the genotypic diversity in the universal phylogeny of life on earth than all other organisms taken together (Pace, 2009). Both the phylogenetic and functional diversity of microorganisms and their numbers and biomass in soil are high, with, e.g. one gram of comparatively unperturbed soil containing up to 10^{10} prokaryotic cells (bacteria and archaeans) representing several thousand of different genomes (Torsvik and Ovreas, 2002).

The use of the ecosystem-services framework for defining protection goals in environmental risk assessment has strong connections to the discussion of the value of specific organisms and biodiversity in general as a base for decisions concerning environmental conservation. The value that can be assigned to the genetic diversity of ecosystems can have different character, as explained above in this section (see also Swift et al., 2004; Cockell, 2005). Values that relate to the system-supporting functions that organisms perform generally in the biosphere or for direct human benefit have been termed their *instrumental* (or *utilitarian*) values. These values of microorganisms are not really a matter of dispute. In contrast, the *intrinsic* value represents the non-use value that biodiversity has on its own, regardless of any human benefits. Clearly, it is not possible to protect (individual) microorganisms at the same levels of attribute and ecological entity as for vertebrates or plants. Although the question whether microorganisms have intrinsic value is largely unresolved, there have been claims that microbes should have increased attention in biodiversity-conservation programmes (Cockell and Jones, 2009; Bodelier, 2011).

It can be expected that the functional capacity of microbial communities in soil is somehow related to the genetic or structural diversity of the total assemblage of microorganisms. The biodiversity–ecosystem function relationship in microbial communities has lately received much attention (e.g. Griffiths et al., 2001; Swift et al., 2004; Fitter et al., 2005; Wertz et al., 2007; Hallin et al., 2009; Philippot et al., 2013; Krause et al., 2014; Singh et al., 2014). This structure/function relationship is quite complex, however, and Ducklow (2008) found that in the information available at that time there were roughly equal numbers of positive, negative or inconclusive relationships between functional properties and diversity of microbial communities. One reason for such variable results, in studies on the biodiversity–ecosystem function relationships, can be the functional redundancy in microbial communities, meaning that if organisms mediating a certain function are inhibited or eliminated, other organisms take over and fill the niche. Functional redundancy can be expected to contribute to the lack of biodiversity–ecosystem function relationships mainly for common properties shared by many of the microbial populations; for specific functions carried out by fewer populations, the relationships are more direct and positive (Ducklow, 2008). Another factor of importance is that only a minority of publications on biodiversity–ecosystem function relationships report results from proper manipulation experiments, while the majority rely on designs where properties of different systems are compared (Krause et al., 2014). Additionally, it may be that although microbial genetic diversity *per se* is not strongly correlated with a particular functional capacity, the sizes of the concerned populations are (e.g. Hallin et al., 2009).

Although the discussion regarding the importance of microbial diversity for ecosystem functioning of soils and the role of functional redundancy goes on, it is clear that loss of microbial diversity can have an impact on function, at least for relatively specific functions performed by few populations (Philippot et al., 2013; Singh et al., 2014). Recently, several studies have suggested that introducing the concept of community-aggregated traits may lead to a better understanding in the future of the role of biodiversity in supporting functions and ecosystem services (Fierer et al., 2014; Krause et al., 2014).

For microorganisms, there is no scientific framework in place for making protection at the population (i.e. species) level possible. The major drawback is that the majority of soil bacteria and archaeans have largely unknown specific properties, since they have not been isolated and studied in pure form. Additionally, just to determine the biogeographical distribution and 'rarity' of a microbial species is a big technical and scientific undertaking. In addition, since bacteria and archaeans grow as

clones, the species concept is arbitrarily applied in these groups. Due to these factors, there is as yet no scientific basis for protecting single microbial species and they are not included in current red-listing of threatened species, with the exception of fungi producing different types of fruiting bodies. In the Opinion of the EFSA PPR Panel on sediment organisms (EFSA PPR Panel, 2015b), 'functional groups' was used as the ecological entity for protection of SPU for microbes across different ecosystem services/specific protection goals.

Even if relationships between genetic microbial diversity in soil and the ecosystem functions they provide are complex and still discussed in the scientific community, clearly there must be some such relationships. At the moment, it seems reasonable for the majority of ecosystems services to use the same general ecological entity of microorganisms to be protected as in the EFSA sediment opinion (EFSA PPR Panel, 2015b), i.e. 'functional groups'. However, for the genetic resources provision and biodiversity support, it is proposed to use the (entire) community as the entity to protect for bacteria, archaeans and fungi since at least some specific functions can only be performed by a restricted subset of the community.

It is proposed to use the diversity, i.e. the variety/richness, as the attribute. Diversity of fungal, bacterial and/or archaeal communities in soil can be described using both structural and functional terms and methods (see Appendix I and Section 9.3 for more details on microbiological tests) and both approaches are potentially useful for describing effects of PPPs on fungal, bacterial and archaeal communities.

For mycorrhizal fungi, although they are obligate symbionts and need to establish a close symbiotic association with plants in order to growth, it is proposed to also protect them at the community level as for other soil microorganisms. This is mainly due to: (i) the absence of an absolute host-specificity; (ii) low species diversity for arbuscular mycorrhizae but high within-species genetic variability and trait plasticity (Koch et al., 2004; Munkvold et al., 2004); (iii) high species diversity for ectomycorrhizal fungi but lack of exhaustive taxonomic information makes the identification at the species level uncertain (Kõljalg et al., 2004); (iv) possible co-occurrence of multiple fungal species able of occupying different niches as well as to the possibility of co-occurring host species having distinct mycorrhizal communities (Dickie, 2007).

5.2. In-soil organisms as drivers in maintaining cultural services

According to MEA (2005), cultural services are those non-material benefits people obtain from ecosystems through spiritual enrichment, heritage values, inspiration, recreation, and aesthetic experiences. Many studies focusing on soil ecosystem services do not address 'cultural services' (Daily et al., 1997; Wall et al., 2004; Lavelle et al., 2006; Barrios, 2007; Weber, 2007); however, *a priori*, cultural services have no greater or lesser value than provisioning, regulating or supporting services. It has to be noted that the intrinsic value of biodiversity and species of conservation concern can be considered a 'cultural service', since the species-based approach to conservation considers biodiversity as a good that has a range of cultural values, such as aesthetic, recreational and existence values.

Soil, as part of the landscape, supports vegetation, and across many cultures has been the source of aesthetic experiences, spiritual enrichment, and recreation. Comerford et al. (2013) associated cultural and spiritual ecosystem services with land and landscape and a 'sense of-place'. Agriculture provides cultural services as some farmers, for example, may maintain field-boundary vegetation or contribute in diversifying landscapes by planting hedgerows, shelterbelts or trees. Some farms also provide accommodation (Dominati et al., 2010), providing places for carrying out recreational activities and nature appreciation. Soil and land provide images that permeate our written and linguistic heritage and our art, such as painting, sculpture, photography, film-making, etc. According to Landa and Feller (2009), soil art combines the diverse roles and functions soil may have, being habitat, growth medium for plants, substrate for architecture, natural filtration system as well as basis for cultural identity. Soil art also explores the aesthetic value of soil with its colours and textures. Soil also plays a direct role in our cultural and religious traditions as a physical material and various cultural uses across the globe (Dominati et al., 2010; Comerford et al., 2013). In addition, soil and agricultural landscapes also play a key role in environmental education. The assessment of cultural services is *per se* difficult, as some contexts elicit aesthetic experiences that have traditionally been called 'scenic beauty', others may elicit different experiences, such as perceived care, attachment and identity (Gobster et al., 2007; Manachini, 2013).

The perception of fulfilled values is very personal and dependent on the social context. For example, significant differences in the perception of the cultural value of agricultural areas have been described for 'farmers', 'naturalists' and 'students', thus demonstrating that cultural services are not absolute values (e.g. Rogge et al., 2007; Natori and Chenoweth, 2008; Tempesta, 2010; Weyland and

Laterra, 2014). Interestingly, if a general rule can be set up, Weinstoerffer and Girardin (2000) see in humans a general attraction for 'diversity, which is source of pleasure, satisfaction or happiness'. The human perception and attraction for nature and biodiversity can also vary among different major groups of organisms. For instance, single individuals and species of plants, insects and other animals are often admired for their beauty. On the other hand, the attraction of bacteria and archaeans is much less obvious and can be more connected to their importance for creating living 'healthy' topsoils which form excellent growth medium for plants. Even the typical 'soil smell' of such healthy soils is perceived as attractive by many. A main reason that prokaryotes have received less attention than other organisms in this respect is that they are microscopic and not visible by the naked eye. Another contributing factor could be the fact that it is not possible for humans to identify with particular species of prokaryotes in the same way as animals or plants.

Soil structure, a result of soil formation processes including the activity by in-soil organisms, has been often taken up by visual and plastic arts (e.g. https://soilarts.wordpress.com/).

In order to help achieving the 'desirable complementary relationship between aesthetic pleasure and ecological health' (van Zanten et al., 2013), it is suggested in the framework of this Opinion to couple the service providing units for in-soil organisms providing cultural values to the SPU for genetic resources and biodiversity.

5.3. In-soil organisms as drivers of nutrient cycling

Soil fauna

Soil fauna is the assemblage of a very diverse group of invertebrates, including e.g. earthworms, arthropods, gastropods, nematodes. Many representatives of soil fauna are important facilitators of organic matter decomposition and nutrient transformation. They can feed on litter material and excrete it partially decomposed as faecal pellets, thus increasing the surface area for microbial activity (Hasegawa and Takeda, 1995). They also act as dispersal agents of fungal spores and bacteria. Moreover, they exert a more direct effect on microbial communities by performing a selective grazing on archaea, bacteria and fungal hyphae (Lummer et al., 2012; Garcia-Palacios et al., 2013), promoting microbial (mainly fungal) succession in decomposing plant material, accelerating decomposition and enhancing nutrient mineralisation (Cragg and Bardgett, 2001; Cole et al., 2005; Ke et al., 2005; Crowther et al., 2012).

Even though microorganisms are directly involved in the biochemical decomposition of organic matter and nutrient transformations, their activity is closely related to the presence and activity of soil invertebrates that act as catalysts of microbial activity and modulators of community composition of microorganisms (Lavelle and Spain, 2005). The role of soil fauna in organic matter decomposition and nutrient turnover can be direct, via feeding on litter material (fragmentation), or indirect, by creating favourable conditions for microbial communities. Many species composing the soil macrofauna (lumbricids, isopods, millipedes, ants, insect larvae) and mesofauna (collembolans, mites, enchytraeids) are actively involved in organic matter breakdown via their feeding activity, contributing to its efficient and fast decomposition and associated nutrient release (e.g. Ketterings et al., 1997; Schrader and Zhang, 1997; Briones et al., 1998; Filser, 2002; Dechaine et al., 2005; Van Eekeren et al., 2008).

'Litter transformers' (e.g. isopods, millipedes and some earthworm species) participate in the early phase of this process, promoting litter fragmentation and influencing microbial dynamics by altering substrate quality when excreting the vegetal material as faeces. They develop external mutualistic associations with microflora by contributing to an increase in substrate surface area accessible to microbial attack and to an increase in substrate pore volume and aeration, thus enhancing the overall microbial resource exploitation (Hassall et al., 1987; Kayang et al., 1996; Maraun and Scheu, 1996; Cotrufo et al., 1998). This may, ultimately, influence nutrient mobilisation rates in the system (Anderson et al., 1983; Teuben and Roelofsma, 1990; Verhoef and Brussaard, 1990).

However, results from microcosm studies reported in the literature on the effects of 'litter transformers' on microbial communities are diverse. Enhanced microbial activity induced by the presence of isopods or diplopods was observed for oak litter (Hanlon and Anderson, 1980; Anderson et al., 1983), black pine (Teuben and Roelofsma, 1990) and on ^{14}C-labelled pondweed (Griffiths et al., 1989). In contrast, a decrease in microbial activity caused by macrofauna was reported for oak litter under high feeding intensity (Anderson et al., 1983), for poplar (Van Wensem and Adema, 1991; Van Wensem et al., 1991, 1992) and for a mixture of deciduous leaves (Vink and Van Straalen, 1999). When litters in different decomposition stages were analysed, contrasting effects were found in experiments with poplar (Van Wensem et al., 1997), black pine (Teuben, 1991) and beech (Maraun

and Scheu, 1996). These results indicate that the type and magnitude of effects depend, among other factors, on the type and status of the substrate, namely its chemical composition and degree of processing by soil fauna.

Effects on nutrient mineralisation may follow similar contrasting trends, and the observed diverging results have been also related to the nutrient status of the litter. To a certain extent, animal activity seems to act as a buffering factor, inducing an element of mineralisation when basal nutrient contents are low and *vice-versa* (Teuben and Roelofsma, 1990; Teuben, 1991). Model simulations revealed that effects of isopods on nutrient pools in decomposing litter were dependent on the litter C:N ratio (Van Wensem et al., 1997), with available carbon and nitrogen levels increasing in the presence of woodlice in litter with medium to high C:N ratios. The role of litter transformers on decomposition is not only affected by the quality of litter or soil organic matter but also by microbial communities and nutrient release. In a microcosm experiment with isopods, millipedes and earthworms, evaluated either as single species or in different combinations, Heemsbergen et al. (2004) demonstrated that litter decomposition, microbial respiration and nitrogen mineralisation were more influenced by community functional dissimilarity (different species with different traits and having different roles in the process) than by species richness *per se*.

Although 'ecosystem engineers' are involved in litter consumption and nutrient cycling, their role in organic matter turnover is also exerted via their burrowing and casting activities, providing habitat for microbes and facilitating the availability of organic substrates, regulating their decomposition activities (e.g. Jegou et al., 2001; Smith and Bradford, 2003; Cole et al., 2006; Frouz et al., 2006; Postma-Blaauw et al., 2006). Brussaard et al. (2007a) postulate that the role of soil macrofauna on water and nutrient use efficiencies in crop areas might be better related to their influence on soil structure. The biogenic structures they produce (soil aggregates and pores) modulate the water and nutrient fluxes, with macroaggregates contributing to the stabilisation of soil organic matter and to the storage of nutrients and their consequent slow release during their decomposition (Jimenez et al., 2003; Brussaard et al., 2007b; Mariani et al., 2007). However, their influence on N mineralisation can be seen beyond the area of the burrows, as found out for *Lumbricus terrestris* by Amador et al. (2006), who observed an increase in soil nitrate in the surrounding of these structures in a mesocosm experiment. The role of earthworms in nitrogen mineralisation seems to depend on the ecological group they belong to and the nutrient source. In a microcosm experiment analysing the influence of different combinations of earthworm species representing different life strategies, Postma-Blaauw et al. (2006) reported enhanced mineralisation of crop residues in the presence of epigeic and anecic species (*Lumbricus rubellus* and *Lumbricus terrestris*, respectively), whereas the mineralisation of SOM increased in the presence of the endogeic species *Aporrectodea caliginosa* in combination with the epigeic species *Lumbricus rubellus*. Both interactions resulted in a reduction in mineral N in soil, possibly due to its immobilisation in microbial biomass. When the endogeic and anecic worms were present, an increase in microbial biomass was also observed with a decrease in total soil carbon. These results demonstrate that the effect of earthworms on nutrient mineralisation depends on the traits of the different species present and can be modified by their interactions. Most land snail species are herbivorous and feed mainly on decaying or half-decayed plant material; some are predators (Burch and Pearce, 1990) and possess a very different feeding strategy compared to, e.g. earthworms. There is no known herbivorous snail species in Europe whose food spectrum is limited to particular plant species and a few snail and slug species are known as pest organisms in ruderal systems, often due to their preference for crop plants that show higher palatability than their wild forms (Kerney et al., 1983; Gosteli, 1996). As for other organisms, some terrestrial snails and slugs can be, thus, considered both non-target organisms and pest species, depending on particular circumstances. Considering only ingestion, the average contribution of terrestrial gastropod species to litter input in temperate ecosystems seems to be lower than for other soil invertebrates (see e.g. Mason, 1970; Jennings and Barkham, 1976; Petersen and Luxton, 1982), although data on environments other than woodlands are scarce. Comparing anatomical and physiological features, Wieser (1978) reported gastropods to be 'both efficient digesters and assimilators', whereas isopods can be considered 'efficient digesters but usually inefficient assimilators', which suggests that gastropods turn over a lower amount of organic material for the same gain of nutrients compared to isopods. Newell (1967) discusses the possible role of terrestrial gastropods in soil formation and states that terrestrial gastropods may have an important function by producing partially digested plant material and modifying their environment during crawling and with their faeces. Faeces and mucus may provide a suitable habitat for the proliferation of microorganisms as a starting point for decomposition processes, which Dallinger et al. (2001) consider to be probably their most important function in nutrient cycling

in temperate ecosystems. Since terrestrial gastropods (mainly snails but also slugs) probably make a significant contribution to the fixation of calcium in the upper soil layer, they may have a strong impact on nutrient cycling in terrestrial ecosystems by diverting fluxes and changing availabilities of macronutrients in terrestrial ecosystems (Dallinger et al., 2001).

Many soil arthropods ingest large amounts of dead organic matter, fungal hyphae and bacteria. Although their role in direct plant-litter decomposition is probably minor, they significantly affect organic matter decay by a range of indirect effects. For example, several studies have shown that, at a moderate density of Collembola, litter enzyme activity, litter respiration and rates of nutrient release increase when compared with litter decomposing in the absence of these animals (Verhoef and Brussaard, 1990). The influence of springtails on nitrogen and phosphorus mineralisation depends on the dominant species involved and their traits, on the climate and type of ecosystem (Cragg and Bardgett, 2001; Filser, 2002).

Interactions between microarthropods and nematodes have been reported to affect soil carbon (C) and nitrogen (N) cycles (Yeates, 2003; Osler and Sommerkorn, 2007). In addition, abundance of total, bacterivorous, and fungivorous nematodes were found to be positively correlated with net N mineralisation rates. Neher et al. (2012) attempted to quantify the relative importance of specific faunal groups in the decomposition of organic matter and for the N availability in soils. Variation in soil N availability and decomposition rates were analysed accounting for the contributions of two faunal communities: nematodes and arthropods. Nematode communities explained between 7% and 12% of the variation in NO_3^- and NH_4^+ availability, indicators of N mineralisation, in disturbed and undisturbed forests. Microarthropod communities explained almost 15% of the variation in decomposition rates in forests. Therefore, alterations in soil food web structure can result in significant changes in decomposition processes (Setala, 2002).

Considering that soil fauna includes a number of identified 'ecosystem engineers', the ecological entity holding different traits in terms of nutrient mobilisation and cycling in agricultural soils is the functional group. However, it is questioned whether defining different functional groups (e.g. anecics worms = vertical burrowers, endogeic worms = burrowing in soil matrix and epigeic worms= surface dwellers) as the ecological entities to be protected would be sufficient to address these key drivers. As illustrated above in the section on the role of species diversity for the long-term performance of functional groups in strongly disturbed agricultural soils, the solely definition of SPU at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long-term performance of the functional role of soil fauna in nutrient cycling of agricultural soils, it is therefore recommended to define the SPU as the abundance/biomass of species belonging to different functional groups.

Soil microorganisms

Soil microorganisms play a dominating role in the degradation of organic matter in soil, which results in mineralisation of C and the essential macronutrients N, P and S (Hussain et al., 2009; Hopkins et al., 2010). The mineral forms can then be further transformed by specific groups of microbes (Prosser, 2007; Hopkins et al., 2010). Since in crop-production soils the plant material is largely harvested and removed, the direct dependence on remineralisation of primary production in field is principally over-run. Resident soil microbial communities, however, still perform critical functions related to mineralisation and transformation of nutrients supplied as organic and inorganic fertilisers (e.g. Ninh et al., 2015).

The nitrogen cycle is particularly relevant to how in-soil organisms increase fertility and is one of the most studied processes. Nitrification and denitrification represent key processes determining the availability and forms of nitrogen (N) in soils. The ability to denitrify is widespread among various microbial taxa, including such phylogenetically diverse groups as bacteria, archaea and eukaryotes (Hallin et al., 2009; Szukics et al., 2010). Conversely, nitrification, including ammonium oxidation and nitrite oxidation, was long believed to be accomplished by a small, specific group of bacteria, until the existence of an archaeal ammonium oxidiser was identified about a decade ago (Hu et al., 2014). Fixation of atmospheric nitrogen by diazotrophic bacteria is a significant N source in rice and legume cultures, and is thus critical for sustainable production in these systems. In rice paddies, it can be performed both by heterotrophs and photosynthetic cyanobacteria (Choudhury and Kennedy, 2004; Wartiainen et al., 2008) and research is ongoing, aiming to increase the use of cyanobacterial inoculants in rice production (Choudhury and Kennedy, 2004; Das et al., 2015). In legume crops, nitrogen fixation is performed by several genera of root-nodule forming bacteria (Graham, 2008). Rhizobium-legume symbioses massively contribute to biological nitrogen fixation entering soil ecosystems (Hussain et al., 2009) with over 100

agriculturally important legumes. Altogether, Rhizobia form symbiotic relationships with an estimated 15,000 legume species. The symbioses between *Rhizobium* or *Bradyrhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of fertiliser-N. It is estimated that N-fixing bacterial symbionts of the legumes can contribute up to 20% of all plant N. Actinorhizal interactions (Frankia-non-legume symbioses) are major contributors to nitrogen inputs in forests, wetlands, fields and disturbed sites of temperate and tropical regions.

Microorganisms also play a central role in the phosphorus cycle. Most agricultural soils contain large reserves of phosphorus. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertiliser is rapidly immobilised soon after application and becomes unavailable to plants. A second major component of soil P is organic matter. Organic forms of P may constitute 30–50% of the total phosphorus in most soils, although it may range from as low as 5% to as high as 95%. To make this form of P available for plant nutrition, it must be hydrolysed to inorganic P. Mineralisation of most organic phosphorous compounds is carried out by means of phosphatase enzymes, such as acid phosphatases. Soil bacteria expressing a significant level of acid phosphatases include strains from the genus *Rhizobium*, *Enterobacter*, *Serratia, Citrobacter*, *Proteus* and *Klebsiella*, as well as *Pseudomonas* and *Bacillus* (Rodríguez and Fraga, 1999).

The provision and the regulation of primary production is one of the most important services delivered by soils. Plant growth and productivity is heavily influenced by the interactions between plant roots and the surrounding soil, including the microbial populations within the soil. Thus, soil microorganisms have a strong impact on plant productivity. The main mechanisms for plant growth promotion driven by microorganisms include suppression of disease (biocontrol), enhancement of nutrient availability (biofertilisation), and production of plant hormones (phytostimulation) (Pereg and McMillan, 2015). The service of pest regulation is indirectly related to the primary production, since such a control limits the loss of plants and plant products.

Plant uptake of water and mineral nutrients from the soil is greatly aided by mutualistic associations with mycorrhizal fungi, which grow into and extend out of the plant roots. Nutritional fluxes are bidirectional (Berruti et al., 2014). Nitrogen acquisition strategies are different in arbuscular mycorrhizae and ectomycorrhizal fungi (Marschner and Dell, 1994; Rebel et al., 2013) but both play a key role in providing plants with phosphorus, which is mainly available in soil as insoluble organic or inorganic forms that make it unavailable to plants (Jones et al., 1998; Smith et al., 2011; Berruti et al., 2014). Arbuscular mycorrhizal fungi have been shown to enhance plant productivity by improving P uptake by plants (Van der Heijden et al., 2006) up to 90%. This is particularly important for legumes, for example, which have a high P-requirement. Also, a functional complementarity has been demonstrated between families of arbuscular mycorrhizae. For instance, Glomeraceae provides protection against fungal pathogens while Gigasporaceae enhances P uptake (Van der Heijden et al., 2008).

It is known that morphological arbuscular mycorrhizae traits are rather well preserved within the same genus (i.e. hyphal length, fungal biomass, number and volume of spores, internal vs external mycorrhizal root colonisation). However, the manifestation of those traits can be quite variable even within one species, being highly dependent from the host plant (and plant community) and the symbiosis established. Among the potentially important ectomycorrhizal fungal response traits influencing their abundance in various communities are preference for N source, exploration morphotype and the deposition of melanin in cell walls (Koide et al., 2014). Fungi that form ectomycorrhizae are not a monophyletic group in contrast to arbuscular mycorrhizae which all belong to the monophyletic group, the Glomales. The ectomycorrhizae can belong to all of the phyla of true fungi (Zygomycota, Ascomycota and Basidiomycota) (Horton and Bruns, 2001). Ectomycorrhizae communities are species rich, however, there is still a lot of uncertainty on the composition of ectomycorrhizae community in terms of number and abundance of species mainly because ectomycorrhizae are not easily manipulated and cultivated in laboratory. One of the main elements of mycorrhizal symbiosis is foraging for nutrients and carbon. The foraging strategy may include: proliferation of hyphae, carbon and nutrient allocation within a mycelium and spatial distribution of the mycelium (internal mycelium for carbon and external mycelium for nutrients) (Olsson et al., 2002). It is well reported in the literature that foraging-related functional traits of hyphae are typically conserved at the genus level (Agerer, 2006; Aguilar-Trigueros et al., 2014), although it is also reported that significant within-species functional variability exists in ectomycorrhizal fungi (Koide et al., 2007).

Due to the complexity of soil fungal communities and the interaction plant-fungi also considering environmental variables, arbuscular mycorrhizae and ectomycorrhizae taxa have not been categorised by using specific criteria but a mix of taxonomic, morphological and physiological characteristics.

So, although these arguments could indicate that 'population' (= species) would be the ecological entity to protect, the variation of trait expression within each arbuscular mycorrhizae species, being highly context-dependent and to the ubiquity of these fungi species in terms of geographical distribution (Davison et al., 2012) and ability to colonise many plant species, makes it difficult to base the risk assessment at population (species) level and rather focus on the functional group arbuscular mycorrhizae. The same conclusion can also be drawn for ectomycorrhizae because, although much has been learned about behaviour, physiological ecology and traits have been measured on individual species, particularly of *Suillus*, *Rhizopogon*, *Paxillus*, *Laccaria*, *Pisolithus* and *Cenococcum*, in laboratory microcosms, meaningful extrapolations to species also depend on trait variability among individuals and populations, as well as on the adequacy of the species concept for fungi. In addition, it is not very clear how factors like host diversity, soil types, organic inputs, disturbance, and succession can affect the structure composition. Moreover, vegetative structures of these fungi (i.e. mycorrhizae and mycelium in the soil) occur largely below ground and are difficult to track and identify (Horton and Bruns, 2001).

Free-living microbes also increase nutrient availability for plants through breakdown of organic matter and releasing mineral nutrients to soil solution. For example, most of the N in soil is contained in complex insoluble polymers, such as proteins, nucleic acids and chitin, which are broken down and mineralised by soil microorganisms, eventually releasing mineral forms of N that are available to plants. In turn, free living N-fixing bacteria are able to fix significant amounts of N, thus contributing to the N budget in many ecosystems. Free-living microorganisms can also contribute to the availability of nutrients to plants by weathering the bedrock via exudation of organic acids and solubilisation of precipitated P. In addition, they can improve plant productivity by suppressing plant diseases, for example through the production of antifungal metabolites by *Pseudomonas* spp.

Soil microorganisms seem to be characterised by a redundancy of functions. However, functional redundancy is considered greater for functions that are performed by a large number of microorganism groups, such as litter decay, than for processes performed only by few specific microbial groups, such as decomposition of specific organic compounds or other processes requiring specific and rare biochemical pathways. In accordance, Singh et al. (2014) suggested that even modest loss of diversity can affect key, specialised functions. Hence, for microorganisms, in case of processes conducted by only a few species, it is more relevant to focus on abundance/biomass of the populations of these specific taxa rather than on biodiversity, respiration rate or biomass of whole microbial communities. For processes conducted by large number of species, such as litter decay, the activity and/or biomass of whole communities may be of interest.

Table 4: Key drivers for the ecosystem service nutrient cycling. Main taxa, exposure routes and examples of species with slow and fast dispersal ability.

Key drivers	Main taxa/ groups	Main exposure routes	Example species
Litter fragmenters/soil organic matter feeders	Isopoda	Oral litter	*Porcellio scaber, Armadillium vulgare Philoscia muscorum, Porcelionides pruinosus*
	Diplopoda	Oral litter	*Julus scandinavicus, Glomeris marginata*
	Collembola	Oral litter/org. matter/microorganisms Contact soil/soil pore water Contact litter/litter water film	*Folsomia quadrioculata, Pseudosinella alba Entomobrya multifasciata*, *Lepidocyrtus cyaneus*
	Oribatida	Oral litter/org. matter/microorganisms Contact soil/soil pore water Contact litter/litter water film	*Oppiella nova, Tectocepheus velatus, Punctoribates punctum, Scheloribates laevigatus*
	Nematoda	Oral litter/org. matter Contact soil/soil pore water Contact litter/litter water film	*Bacterial feeders* (Rhabiditida) *Fungal feeders, ominivorous feeders* (e.g. Dorylaimida, Diplogasterida)
	Enchytraeidae	Oral soil Oral litter/org. matter/microorganisms Contact soil/soil pore water Contact litter/litter water film	*Enchytraeus albidus*
	Lumbricidae	Oral soilOral litter Contact soil/soil pore water Contact litter/litter water film	Anecic worms: *Lumbricus terrestris, Aporrectodea longa* Endogeic worms: *Aporrectodea caliginosa, Aporrectodea rosea* Epigeic worms: *Lumbricus castaneus*

Key drivers	Main taxa/ groups	Main exposure routes	Example species
Bacteria and fungi feeders,		Contact soil/soil pore water Contact litter/litter water film	
Microorganisms	Bacteria	Contact soil/contact soil pore water	
	Mycorrhizal fungi	Contact soil/contact soil pore water	
	Fungi	Contact soil/contact soil pore water	

5.4. In-soil organisms as drivers of pest and disease control

This section treats the ability of in-soil organisms to act as natural competitors, predators, parasites or antagonists, and thereby as biological control agents for pest species or plant diseases. According to Cook et al. (1996), pests are organisms at population densities that cause death or injury, or constitute a nuisance to crops, livestock, pets, people, or the environment. Pest refers to weeds, plant parasitic nematodes, arthropod pests of plants, animals, plant pathogenic viruses, prokaryotes (including bacteria, mycoplasma-like organisms (MLOs), and spiroplasmas), and fungi. Disease is a process that results from a compatible interaction between virulent pathogen and susceptible plant. Plant disease refers to infectious diseases caused by plant pathogenic viruses, viroids, bacteria, mycoplasma-like organisms (MLOs), spiroplasma, fungi, and nematodes. Diseases, however, can also be caused by abiotic factors such as unfavourable soil properties, fertility imbalances, moisture extremes, temperature extremes, chemical toxicity, physical injuries (Kennelly et al., 2012).

Pest control has often been highlighted as an important ecosystem service provided by biodiversity and one that is threatened by modern agricultural practices (Wilby and Thomas, 2002). The natural enemies of insect pests are responsible for about 50–90% of the biological pest control occurring in crop fields (Martin et al., 2013). Soils provide habitat to beneficial species that regulate the composition of communities and thus can prevent proliferation of herbivores and pathogens. This service depends not only on the health of the soil, including its abiotic properties, but also on the biological processes driving inter- and intraspecies interactions (symbiosis, competition, host–prey associations) (Aislabie and Deslippe, 2013). A healthy soil community has a diverse food web where beneficial organisms can contribute to suppressing pests and disease-causing organisms through competition, predation, and parasitism. Evidence from natural systems shows that low diversity of an ecosystem can be associated with a higher vulnerability to pests due to altered top-down and bottom-up control mechanisms. In agricultural fields, for example, the soil functioning is modified and, as a consequence, its equilibrium can be altered leading to outbreaks of crop pests (Turbé et al., 2010).

Soil fauna

As a rule, all species of animals are regulated by other living organisms (antagonists) that are not under manipulation by man but occur naturally in crop environments.

Microarthropods and annelids have been shown to contribute significantly to the ecosystem service 'pest and disease control'. Especially the activity of soil fauna in the control of soil-borne phytopathogenic fungi and their mycotoxins has been investigated (e.g. Schrader et al., 2013). The successful control of fungal biomass and the reduction of deoxynivalenol of *Fusarium*-infected dead organic matter has been demonstrated for the earthworm species *Aporrectodea caliginosa* and *Lumbricus terrestris* (Oldenburg et al., 2008; Wolfarth et al., 2011a,b) and for the collembolan *Folsomia candida* and the nematode *Aphelenchoides saprophilus* (Wolfarth et al., 2013, 2015). Sabatini and Innocenti (2001) could show that the selective feeding activity of the collembolan species *Onychiurus armatus* and *Mesaphorura krausbaueri* on pathogenic fungi of millet and wheat kernels significantly reduced the severity of the disease complex in winter cereals.

Also, the interaction between the activity of the grazing collembola *Proisotoma minuta* and *Onychiurus encarpatus* and those of three biocontrol fungi were studied for suppression of *Rhizoctonia solani* on cotton (Curl et al., 1988; Lartey et al., 1994). Interestingly, all combinations of collembola and fungi inoculations provided more effective disease suppression than the fungal agents used alone. It has been argued that such effective control mechanisms result from the combined preference of soil fauna species for some phytopathogenic fungi, their aversion for other fungal species acting as biocontrols and the direct parasitism of the fungus by other agents.

Nematodes within agroecosystems provide numerous ecological services and economic benefits for pest and pathogen control. Predatory, entomogenous, and entomopathogenic nematodes (EPNs) and omnivorous nematodes consume insect pests, fungal and bacterial feeders control populations of fungal and bacteria pathogens of plants, as in the case of *Aphelenchus* spp. (Lagerlof et al., 2011), while plant-feeding nematodes can also affect weeds. Entomogenous nematodes, i.e. nematodes associated (often parasitically) with insects, are a group of insect-killing nematodes. Some species are currently used for biological control or Integrated Pest Management (IPM). EPNs live parasitically inside the infected insect host, and so they are termed as *endoparasitic*. They carry bacteria that infect many different types of insects living in the soil, like the larvae of moths, butterflies, flies and beetles, as well as adult grasshoppers and crickets. EPNs have been found all over the world and in a range of ecologically diverse habitats. Nine families of nematodes (Allantonematidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinernematidae and Tetradonematidae) include species that attack insects and kill or sterilise them, or alter their development. The most commonly studied entomopathogenic nematodes are those that can be used in the biological control of harmful insects: the members of Steinernematidae and Heterorhabditidae families. Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control agents (Kaya and Gaugler, 1993). They are soil-inhabiting organisms and can be used effectively to control soil-borne insect pests, but are generally not effective when applied to control insects in the leaf canopy. When considered as a group of nearly 30 species, each with its own suite of preferred hosts, entomopathogenic nematodes can be used to control a wide range of insect pests, including a variety of caterpillars, cutworms, crown borers, grubs, corn root worm, cranefly, thrips, fungus gnat and beetles. Dozens of different insect pests are susceptible to infection by entomopathogenic nematodes, yet no adverse effects have been shown against beneficial insects or other non-target animals in field studies (Georgis et al., 1991; Akhurst and Smith, 2002). In addition, EPN are often important for the potential control of alien insect species (Landi et al., 2009). Certain nematodes can also parasitise spiders, leeches, annelids, crustaceans and molluscs.

EPNs can be used as biological control agents to suppress a variety of economically important insect pests, especially in IPM and integrated production (IP) systems (Grewal, 2002; De Nardo and Grewal, 2003). The species that have been most studied in this context are those that have been introduced as biopesticides, while few data are available for native EPNs. Duncan et al. (2013) recorded a strong negative correlation between the density of native EPNs and the abundance of the root weevil pest of citrus, *Diaprepes abbreviatus*, highlighting that EPNs can have an important role in the control of the population of this insect pest. Indeed, when evaluating the potential of the use of chemicals integrated with biological control agents (e.g. EPNs), the International Organization of Biological Control (IOBC) developed a sophisticated approach based on a tiered hierarchy made up of threshold values for lethal and sublethal effects on non-target antagonists (Manachini, 2013). However, some synergy of EPNs used together with chemical pesticides has been recorded. Control of larvae of *Diabrotica virginifera* was enhanced by such combination, resulting in a synergistic response and an increase in expected mortality of 24%; the combined effect of the insecticides plus EPNs was greater than either product applied on its own (Nishimatsu and Jackson, 1998). The mechanism for increased nematode efficacy when used together with chemical pesticides is not fully known, although it has been suggested that it could be due to a reduction in the host insect's immunity and activity even at sublethal dosages (Manachini, 2013). On the other hand, Campos-Herrera et al. (2008) have shown that natural EPN populations isolated from crop fields appeared less active against *Galleria mellonella* than those isolated from natural areas and field edges, suggesting that agronomic management could affect their natural activity, reducing their virulence and reproductive potential.

In order to support the long-term performance of the functional role of soil fauna in control of pest and pathogens in agricultural soils, it is recommended to define the SPU as the abundance/biomass of population of microarthropods and earthworms as fungal pathogen controller, while it is recommended to define the SPU as the abundance/biomass of nematode species belonging to different functional groups. Nematodes can be allocated to functional groups to condense information efficiently and to determine their contribution to ecosystem processes, e.g. feeding groups as bacterivores, fungivores, plant feeders, vertebrate and invertebrate pathogens, carnivores or omnivores (see e.g. Bongers and Bongers, 1998). These functional groups could be used as indicators to interpret the nematodes' contribution to the ecosystem services 'pest and disease control'. Additionally, grouping according to the c-p scale (see Appendix A) could help to interpret changes in the community structure and to indicate a decrease in diversity and ecosystem stability. Regarding soil microarthropods and earthworms, the species specific preference for fungal pathogens require the definition of SPU at the level of abundance/biomass of populations.

Soil microorganisms

Agricultural systems host numerous microorganisms with the ability to control populations of pests and diseases (Persmark et al., 1995; Kasiamdari et al., 2002; Barrios, 2007; Stewart et al., 2010). These exert not only control under natural conditions, but also represent a resource that can be tapped for isolates for development of biological pest- and disease-control products. Microbial biocontrol agents include natural enemies and antagonists of pests and pathogens (Cook et al., 1996). Beneficial species include microbes that support plant growth through increasing nutrient availability and by outcompeting invading pathogens (Aislabie and Deslippe, 2013). Some microbes isolated from soil and other habitats have been developed into biocontrol agents and subsequently marketed as biocontrol products. Examples are antagonistic bacteria and fungi used against fungal plant diseases (Whipps and Gerhardson, 2007), pathogens of pest nematodes (Dong and Zhang, 2006; Wilson and Jackson, 2013), and entomopathogenic fungi and bacteria (Inglis et al., 2001). Species reported to act as biocontrol agents in compost-amended substrates include bacteria, such as *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum* and *Pseudomonas* spp., and fungi such as *Penicillium* spp., *Gliocladium virens* and several *Trichoderma* spp. (Litterick et al., 2004). These beneficial microorganisms can be released from the compost or the compost may provide nutrients that stimulate the proliferation of antagonistic bacteria and fungi in the rhizosphere (Noble and Coventry, 2005; Green et al., 2006). Four main mechanisms of suppression by the beneficial microbe of the pathogen have been described: competition for nutrients; antibiotic and enzyme production; parasitism and predation; and enhanced resistance to plant diseases (both induced systemic resistance and systemic acquired resistance). Several or all these different mechanisms of disease suppression may occur simultaneously through the activity of one or more beneficial microorganisms present in disease-suppressive soil or composts. For example, several different *Trichoderma* spp. can compete with pathogens for nutrients and space, exhibit antibiosis, parasitise the pathogen and elicit induced plant resistance. Fungi are the predominant pathogens of insects and play a significant role in the natural regulation of soil-dwelling pests. Insecticidal toxins are produced by most entomopathogenic fungi during pathogenesis. After successfully penetrating the insect cuticle, the fungi enter the haemocoel where they have to overcome insect immune responses in order to colonise and to kill the host. Generally, these toxins are bioactive secondary metabolites secreted during growth inside the insect.

There is no doubt that soils harbour a high diversity of microorganisms that contribute to biological population regulation and more specifically to natural control of pests and diseases. For practical reasons, however, it is extremely difficult to estimate the total range of this control activity, and the contribution of different microbial groups and species. For example, although there have been estimates of the population densities in soil of the marketed biocontrol entomopathogens *Metarhizium* spp. (Bidochka et al., 1998; Schneider et al., 2012) and *Bacillus thuringiensis* (Eskils and Lövgren, 2011; Guidi et al., 2011), very little is known regarding their effect on insect populations under natural conditions.

The natural plant disease-control effect is for some diseases manifested as a general soil suppressiveness and it has been known for a long time that certain soils are suppressive to specific soil-borne plant pathogens (Bidochka et al., 1998; Weller et al., 2002; Termorshuizen and Jeger, 2008). It has been demonstrated that antagonistic microorganisms play a decisive role for this suppressiveness, and for some diseases, the biological basis and contributing microbial groups have been at least tentatively identified (Weller et al., 2002; Mendes et al., 2011).

Mycorrhizal fungi have also been reported among the groups of microorganisms showing antagonism to pathogens, showing the potentials for use as biocontrol agents for soil-borne diseases. The role of mycorrhizae in disease control has been observed both in arbuscular mycorrhizae and in ectomycorrhizal associations. The most relevant aspects of ectomycorrhizae that make them efficient for plant-disease control are: (i) efficient competition with the ubiquitous soil microflora, (ii) root colonisation and mycorrhiza formation at rates faster than the pathogen invading the roots, (iii) suppressive action against most pathogenic species. For arbuscular mycorrhizae, the possible mechanisms involved in biocontrol are: i) enhanced plant nutrition, ii) biochemical changes in plant tissues, iii) anatomical changes, iv) alleviation from stresses predisposing plants to disease, (v) microbial changes in the rhizosphere (mycorrhizosphere), (vi) induced changes to the root-system morphology, (vii) direct competition between the fungi and the pathogens for physical space or resources, and (viii) induction of systemic resistance (Harrier and Watson, 2004; Mukerji and Ciancio, 2007).

Regarding the entity to protect for these fungal groups, as mentioned above in Section 5.3 (service of nutrient cycling), the high range in trait expression within arbuscular mycorrhiza species, highly dependent on the plant they establish the symbiosis with, and the high trait variability between

individuals in the case of ectomycorrhizae, as well as the lack of adequacy of the species concept for this fungal group, makes it appropriate to base the risk assessment at the functional group level (arbuscular mycorrhizal and ectomycorrhizal fungi).

Table 5: Key drivers for the ecosystem service pest control. Main taxa, examples of species, example of traits determining dispersal and exposure routes.

Key drivers	Main taxa	Main exposure routes	Example species
Pest pathogen competitors and suppressors	Nematodes Microorganisms (e.g. fungi and bacteria)	Contact soil/contact soil pore water	Nematodes: Mermithidae *Heterorhabditis* spp., *Steirnenema* spp. Fungi: *Bauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* Bacteria: *Bacillus thuringiensis, Pasteuria penetran*
Toxin dispersal antagonists	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: *Lumbricus terrestris, Aporrectodea longa*
	Collembola	Oral litter/org. matter Contact soil	Collembolans: *Folsomia quadrioculata, Pseudosinella alba*, *Entomobrya multifasciata*, *Lepidocyrtus cyaneus*
Plant pathogen competitors and suppressors	Arbuscular and Ectomycorrhizae, bacteria and fungi	Contact soil/contact soil pore water	

5.5. In-soil organisms as drivers of natural attenuation

Natural attenuation, also called intrinsic bioremediation, is an ecosystem service that helps keep the soil clean and suitable for, e.g. production of food and drinking water (National Research Council, 2000; Rittmann, 2004) and contributes to maintaining a healthy habitat for in-soil organisms, which support other ecosystem services (van Wijnen et al., 2012). The latest two definitions of natural attenuation are the following:

> ... 'Reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by more active methods. The natural attenuation processes that are at work in such a remediation approach include a variety of physical, chemical, and biological processes that, under favourable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. These *in situ* processes include biodegradation; dispersion; dilution; sorption; volatilization; radioactive decay; and chemical or biological stabilization, transformation, or destruction of contaminants'. (USEPA, 1999)

Three main types of process are involved in natural attenuation: (i) transport processes including advection, dispersion, diffusion and sedimentation; (ii) phase-transfer processes being responsible for movement between compartments such as sorption and volatilisation; and (iii) transformation processes being the only ones that effectively reduce the mass of contaminants whereas transport processes mainly affect concentration and exposure. Chemical alteration of contaminants may be due to both abiotic (e.g. dissolution, complexation, hydrolysis, precipitation, oxidation, and reduction) or biotic processes (e.g. biodegradation) (Ouvrard et al., 2013).

Soil fauna

Earthworms enhance soil aeration and moisture and contribute to improve its nutritional status via their activity as ecosystem engineers, within their sphere of influence (the drilosphere, including their burrows and casts). These earthworm activities are often limiting factors for soil microorganisms responsible for the biodegradation of organic compounds (Hickman and Reid, 2008). In this way, earthworms can directly influence microbial communities playing a role in the natural attenuation of chemicals in soil. This has been shown for PAHs where the presence of earthworms (*E. fetida*, *E. andrei* and *L. rubellus*) enhanced the aerobic degradation of PAHs in soil (Ma and Imerzeel, 1995;

Eijsackers et al., 2001; Contreras-Ramos et al., 2006, 2008). This occurred mainly not only through their bioturbation activity (increasing soil aeration, facilitating the attack by microorganisms promoting aerobic degradation) but also via their ability to change the structure, biomass, and functioning of soil microbial communities (Sheehan et al., 2008; Natal-da-Luz et al., 2012), which may indirectly stimulate PAH biodegradation, predominantly dependent on microbial activity (Weissenfels et al., 1992; Shuttleworth and Cerniglia, 1995). However, the role of earthworms on PAH degradation is not always evident since the rate of PAH biodegradation is highly variable and dependent not only on PAH structure but also on the intimate association with the soil matrix, determining bioavailability, and on the composition and activity of soil microbial communities (Shuttleworth and Cerniglia, 1995).

The ability of earthworms to interact with microorganisms involved in pesticide degradation was shown by Liu et al. (2011) when the presence of *Aporrectodea caliginosa* stimulated an increase in activity and abundance of microbes degrading the herbicide MCPA, as well as an increase in the expression of tfdA-like genes (genes encoding oxygenases initiating aerobic MCPA degradation) on their burrows and on the 0–5 cm soil. Increased herbicide mineralisation catalysed by earthworms has also been shown for atrazine (e.g. Meharg, 1996), 2,4-D, carbofuran and metribuzin (Mallawatantri et al., 1996), and isoproturon and dicamba (Gevao et al., 2001). However, opposite results have been shown by other authors (see Hickman and Reid, 2008 for a more detailed analysis of the studies performed), especially in terms of compound sorption and release and the consequent effects on mineralisation. Despite these contradictory results, there is evidence supporting the role of earthworms as agents of biodegradation of organic compounds, especially by creating favourable conditions for the activity of microbes responsible for their degradation. These types of interactions are to be assumed also for other groups of soil fauna enhancing microbial activity, e.g. microarthropods, even if no specific study on natural attenuation in terrestrial ecosystems by soil fauna other than earthworms is known.

Soil microorganisms

Microbial degradation of chemical compounds in the environment is an important route for the removal of these compounds. The process of natural attenuation, i.e. processes driven naturally by indigenous soil microorganisms leading to dissipation through biological transformation, mainly depends on microorganisms that attack the pollutants enzymatically and convert them to innocuous products. The abundance of microorganisms and their high growth rates allow them to evolve quickly and to adapt rapidly to environmentally changing conditions (Diaz, 2004). Natural attenuation processes carried out by microorganisms can be divided into three general categories: 1) the target compound is used as a carbon source; 2) the target compound is enzymatically attacked but is not used as a carbon source (co-metabolism); and 3) the target compound is not metabolised at all but is taken up and concentrated within the organism (bioaccumulation). Although fungi participate in all three categories, they are often more proficient at co-metabolism and bioaccumulation than at using xenobiotics as sole carbon sources.

Bacterial activity is the major process involved in the hydrolysis of organic pollutants. Extracellular enzyme activity is a key step in degradation and utilisation of organic polymers. Hydrolytic enzymes disrupt major chemical bonds in the toxic molecules, which may result in the reduction in their toxicity. This mechanism is effective for the biodegradation of oil spill, organophosphate, organochloride and carbamate pesticides, and especially performed by soil bacteria belonging to the genera *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Micrococcus* (Porto et al., 2011). Mixed microbial communities are usually more efficient in biodegradation because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas.

Most filamentous fungi are unable totally to mineralise aromatic hydrocarbons; they only transform them into products of decreased toxicity and increased susceptibility to decomposition by bacteria, suggesting that the interaction among fungi and bacteria is profitable for petroleum-hydrocarbon mineralisation (Joutey et al., 2013). For example, ligninolytic fungi, such as the white rot fungus *Phanaerochaete chrysosporium*, can degrade several xenobiotics, such as pentachlorophenol and dioxin, under co-metabolic conditions (Aislabie and Deslippe, 2013). However, fungi are also an important part of degrading microbiota because, like bacteria, they metabolise dissolved organic matter. Fungi are more efficient than bacteria especially in breaking down more recalcitrant material, like the natural polymeric compounds (e.g. lignin) thanks to extracellular, multi-enzyme complexes. They are also able to colonise and to penetrate substrates rapidly, and to transport and redistribute nutrients within their mycelium by means of their hyphal systems.

Both ectomycorrhizae and arbuscular mycorrhizae have shown the ability to detoxify toxic substances. It has been demonstrated that the process of remediation carried out by mycorrhizal fungi in conjunction with

other microorganisms is usually faster and more efficient. Both the filamentous fungus, *Cunninghamella echinulata* and the bacterium, *Sphingomonas paucimobilis* have been used in conjunction with arbuscular mycorrhizae for the remediation of a soil polluted with petroleum hydrocarbon (Chibuike, 2013).

For natural attenuation to occur, microbes with the appropriate biodegradative ability must be present in sufficient numbers. This will depend in part on how long they have been exposed to the contaminant. The role of the community of indigenous microbiota is considered essential for an efficient natural attenuation of contaminated soils, because no single microbe has the metabolic potential to degrade all contaminants.

The reasons presented before to consider the 'functional group' as the ecological entity to protect regarding both arbuscular mycorrhizal and ectomycorrhizal fungi for the ecosystem services 'nutrient cycling' and 'pest and disease control' are also valid for this ecosystem service 'natural attenuation'.

Table 6: Key drivers for the ecosystem service natural attenuation. Main taxa, examples of species, example of traits determining dispersal and exposure routes.

Key drivers	Main taxa	Main exposure routes	Example species
Soil non-arthropod invertebrates	Lumbricidae	Oral soil Oral litter Contact soil and soil solution	Anecic worms: *Lumbricus terrestris, Aporrectodea longa* Endogeic worms. *Aporrectodea caliginosa*
	Enchytraeidae	Oral soil Contact soil and soil solution	*Enchytraeus* sp., *Fridericia* sp.
Microfauna	Nematoda	Contact soil and soil solution	
Microorganisms	Bacteria	Contact soil/contact soil pore water	*Bacillus*, *Pseudomonas*, *Arthrobacter* and *Micrococcus* sp.
	Mycorrhizal fungi	Contact soil/contact soil pore water	
	Fungi	Contact soil/contact soil pore water	*Phanaerochaete chrysosporium*

5.6. In-soil organisms as drivers of soil structure formation and water retention

Soil structure consists of soil aggregates and the resulting pore spaces between them. One of the most relevant ecosystem services for the formation and stabilisation of soil structure is soil aggregation (Rillig et al., 2015). The extent of aggregation, the stability of the soil aggregates and the pore space are considered important parameters when evaluating soil structure (Shukla, 2014). Soil structure is a key feature for plant productivity since: (i) it improves soil fertility, increasing agronomic productivity, enhancing porosity and decreasing erodibility; (ii) it can affect plant growth by influencing root distribution and the ability to take up water and nutrient; (iii) it enhances oxygen, water infiltration and water storage (Bronick and Lal, 2005).

Soil fauna

The importance of soil faunal activity for the formation of soil biophysical structures and for the development of soil horizons has been recognised since it was reported by Darwin (1881) in his book 'The formation of vegetable mould, through the action of worms, with observations on their habits'. There he stated that 'Worms have played a more important part in the history of the world than most persons would at first suppose. [. . .] The plough is one of the most ancient and most valuable of man's inventions; but long before he existed the land was in fact regularly ploughed, and still continues to be thus ploughed by earth-worms'. This was probably the first time that the concept of 'ecosystem engineers' was presented to the general public, even if the term was not defined until 1996. In the characterisation by Jones and co-authors, ecosystem engineers 'directly or indirectly modulate the availability of resources (other than themselves) to other species by causing physical state changes in biotic or abiotic materials. In so doing, they modify, maintain, and/or create habitats' (Jones et al., 1996).

Earthworms are not the only group of in-soil organisms acting as ecosystem engineers in the soil compartment by modulating the availability of resources to other organisms or modifying their habitat. In fact, it is a feature of in-soil organisms in general to be strongly influenced by the characteristics of the medium soil around them, but in turn to be able to modify it to some extent according to their particular requirements – also termed 'niche construction' (Weigmann, 1998; Odling-Smee et al.,

2003). Nevertheless, the outcome of the activity of soil micro- and mesofauna is not as remarkably visible at larger scales as the results of earthworms, ants or termites burrowing and 'engineering' the soil, but it has recently become a matter of attention (e.g. Lehmann and Rillig, 2015; Maaß et al., 2015). The definition of ecosystem engineers is therefore not fully straightforward. Soil micro- and mesofauna are not able to burrow in the mineral soil horizons, and are largely confined to pre-existing voids in litter and surface soil horizons, especially if the soil is compacted (Lee and Foster, 1991; Roithmeier and Pieper, 2009).

The processes that can be allocated to in-soil organisms changing the physical environment around them are illustrated in Figure 4 below by Jones et al. (2006), pointing at two fundamental pathways highly interrelated: assimilation/dissimilation (uptake, metabolism) and physical ecosystem engineering. The authors conclude that 'soils and sediments are probably the most highly physically engineered of

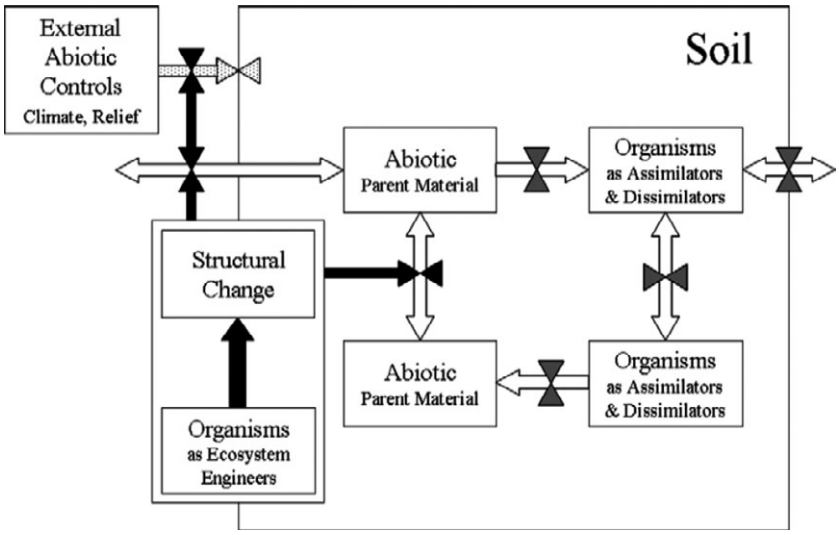

Figure 4: Organismal influence on soil processes, with factors (abiotic, ecosystem engineers and assimilators/dissimilators) in compartments. White arrows: energy and material flows between the compartments under external abiotic control (dotted bow tie). Black arrows: physical ecosystem engineering changes the physical soil structure and influence assimilatory- and dissimilatory-related flows (gray bow tie), including biogeochemical processes (black bow tie). Modified after Jones et al. (2006), copyright Elsevier

all environments'.

Very comprehensive review papers have been published on the role and importance of soil organisms for the shaping of the soil physical environment and for processes of soil-horizon formation and hydrological properties. Please refer for details on the reported studies to the work of Lavelle (2002) and Lavelle et al. (2006), Bottinelli et al. (2015), Berke (2010), Blouin et al. (2013) and Bertrand et al. (2015). Some general patterns will nevertheless be outlined below.

It is important to note that the in-soil organisms' community shapes its environment at different spatial scales (Anderson, 2000; Lavelle, 2002), according directly to their action range and indirectly to the integrated structure/process on the next level. Moving up the hierarchy, successive levels might concern the same processes, but with slower dynamics and covering larger areas (Anderson, 1988).

The smallest scale relates to effects of microbial biofilms on microaggregates or microtubules by fungal hyphae (see chapter below for more detail about soil microorganisms), followed by micro and mesofauna. The effects of small invertebrates, like enchytraeids or microarthropods, producing microgranular pellets structures in the upper soil centimetres are well documented, as are the effects of the three major groups of soil ecosystem engineers (scale 3) – ants, termites and earthworms. Large diversity of biogenic structures will provide the 4th scale, while scale 5 comprises effects of soil invertebrate engineers at the landscape scale, which have also been described (Jones et al., 2012). Table 7 provides a summary of the contribution of soil fauna to ecosystem services related to the physical soil structures.

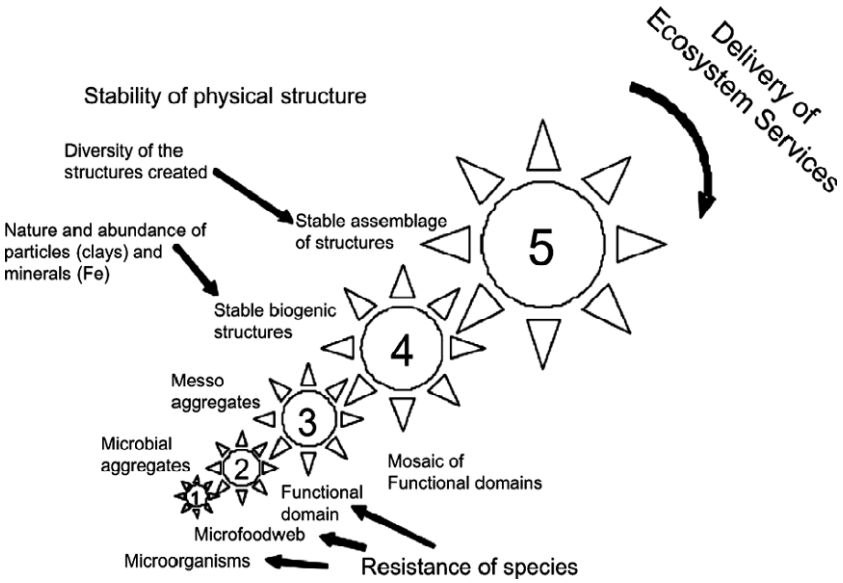

Figure 5: Self-organising systems in soils at different scales from microbial biofilms to the landscape. The stability of delivery of ecosystem services at scales > 5 is supported by the resistance of species to disturbances and/or the stability of physical structures (from Lavelle et al., 2006, copyright Elsevier)

Table 7: Contributions of soil invertebrates to the provision of ecosystem goods and services by soils (after Lavelle (2002) modified)

Service types	Goods/ services	Ecosystem process	Invertebrate contribution	Indicator of soil fauna contribution
Production	Water supply	Infiltration and storage of water in soil pore systems	Building and maintenance of stable porosity through bioturbation and burrowing	Proportion and arrangement of biogenic structures in soil Water-holding capacity Water infiltration dynamics
Support	Soil formation	Pedogenesis	Bioturbation Surface deposition Particle selection	Development of horizons DNA and near infrared spectroscopy analyses in biogenic structures
Regulation	Flood and erosion control	Regulation of water runoff Infiltration and storage of water in soil	Creation of surface roughness by biogenic structures Building and maintenance of stable porosity through bioturbation and burrowing	Production of biogenic structures Soil and humus morphology

Of particular importance in agricultural fields is the ecosystem service 'soil formation and water retention' since it addresses issues of solute transports in soils and possibly the movement of PPP in the soil profile. Soil fauna has been reported to increase the hydraulic conductivity of soils, to increase preferential flow but also to reduce run-off and to increase soil porosity (e.g. Shipitalo et al., 1994; Friend and Chan, 1995; Pieper et al., 2008; Bailey et al., 2015; Laine-Kaulio et al., 2015). The activity of soil fauna affects the formation and permanence of hydrophobic patches in soil that promote preferential flows. Earthworms have being proposed as a means of reclaiming heavily hydrophobic soils (Ritsema and Dekker, 2000; Mueller and Deurer, 2011).

It is important to discriminate between the actions of different functional groups of soil fauna when characterising their effects and evaluating studies with contrasting outcome. Without prejudice to the difficulties of defining 'true' functional groups, behavioural and feeding traits of species groups determine the structures impacted and/or created in soils. Anecic worms do burrow vertically in soils and live in almost permanent burrows even open to the soil surface, facilitating direct solute transport to deeper soil layers and reducing run-off events. Earthworms living mostly in the upper 15 cm of the soil, so called endogeics, refill the horizontal burrows they have channelled with the soil they have processed and therefore increase the water-holding capacity of soils and prevent the formation of stable hydrophobic patches. Even if not very well studied, the impact of soil micro- and mesofauna on soil structures is deemed to resemble at smaller scales the activity of endogeic worms (e.g. Van Vliet et al., 1998).

Collembola contribute to soil aggregation. The soil in the hyphal compartment shows greater soil aggregation with larger mean weight diameter when Collembola are present, and a similar result was found in the presence of arbuscular mycorrhizal fungi compared to control treatments. Moreover, the combined presence of both arbuscular mycorrhizal fungi and Collembola results in a non-additive increase in soil aggregation. The study by Siddiky et al. (2012) clearly indicates that Collembola can enhance soil aggregation and can partially complement effects of arbuscular mycorrhizal fungi, and that these effects are independent of roots.

Degraded soil structure in agricultural soils might result in soil compaction, defined as a process that rearranges soil grains, resulting in decreased void space and increased bulk density (Soil Science Society of America 2008). Compaction accounts for around 68 million hectares of degraded soil worldwide (Flowers and Lal, 1998). Compaction might result not only from, e.g. intensive farming with heavy machinery, but also from animal grazing (Ferrero and Lipiec, 2000; Gayle et al., 2005). Among others, compacted soils are characterised by increased soil strength and less interconnected pores (Schjønning et al., 1998; Hamza and Anderson, 2005), by reduced soil aeration and drainage capacity (Larink et al., 2001). Plants suffer from hampered rooting ability and spatial access to nutrients and water (Dannowski, 1994; Larink and Schrader, 2003; Kuchenbuch and Ingram, 2004) and soil fauna is faced with the deterioration of living conditions through the loss of habitable space, with increased injuries and possible death (Brussaard and van Fassen, 1994; Horn et al., 1994; Larink and Schrader, 1999, 2003). It has been reported that the abundance and activity of collembolan and enchytraeids negatively correlates in the field with increased soil bulk densities, probably due to a reduction in coarse pores as habitable pore space (Heisler and Kaiser, 1995; Schrader and Zhang, 1997; Langmaack et al., 1999; Dittmer and Schrader, 2000; Larsen et al., 2004).

As a feature of the ecosystem service 'soil-structure formation' by soil animals, burrowing soil fauna may, however, loosen compacted soil through bioturbation, e.g. by earthworms (Barros et al., 2001; Larink et al., 2001; Schrader et al., 2001). Also soil mesofauna as Enchytraeidae are known to improve pore structure and connectivity (Didden, 1990; Van Vliet et al., 1998), positively affecting gas exchange, water conductivity, and plant root growth through the soil (Schrader et al., 1995; Van Vliet et al., 1998; Larink and Schrader, 2003).

Even if, 'extrapolations of faunal activity detected at the microhabitat scale to the level of watershed without an explicit consideration of the multiplicity of structures may facilitate a conclusion of the functional redundancy of soil fauna' (Heneghan and Bolger, 1998), and considering soil micro- and mesofauna to have a scarcity of 'ecosystem engineers', the ecological entity holding different traits in terms of soil formation is the functional group. The attribute we are protecting – and in this case also measuring – is the abundance/biomass of species belonging to different functional groups. The difficulties in defining functional groups originate from the scarce knowledge in attributing specific function to species of microarthropods (compared to, e.g. earthworm species). Nevertheless, also microarthropods have different traits regarding their role in soil processes.

Regarding non-arthropod invertebrates belonging to the ecosystem engineers, the ecosystem service 'soil formation' including retention function is provided by anecics (= vertical burrowers) and endogeic (= burrowing in the soil matrix) and epigeic (= dwelling in the organic matter at the surface) earthworm functional groups. It is however questionable, if the ecological entity to be protected should be defined as a functional group. Having often under field conditions in agricultural landscapes only single to few species belonging to, e.g. anecic groups, the entities to be protected would be the populations of these earthworm species. Species diversity holds a key role for the long-term performance of functional groups in strongly disturbed agricultural soils. In these environments, the defining SPU solely at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long-term performance of the functional role of soil fauna in soil structure formation, it is therefore recommended to define the SPU as the abundance/biomass of species belonging to different functional groups.

Soil microorganisms

Microbial communities can be considered as architects of soils. Soil aggregation influences virtually all nutrient cycling processes and soil biota. Microorganisms are key organisms in aggregate stabilisation and in decomposition of plant litter. Both fungi and bacteria contribute to stabilisation of soil aggregates through deposition of extracellular polysaccharides binding soil particles and formation of degraded, aromatic humic materials (Umer and Rajab, 2012). The formation of humic substances by soil microorganism is catalysed by microbial exo-enzymes (Guggenberger, 2005). Fungal hyphae improve

aggregate stability by reorientation of clay particles, binding particles with extracellular polysaccharides, and enmeshing particles. Hyphae also enmesh microaggregates to form macroaggregates suggesting that aggregation increases with hyphal density.

Within the huge variety of soil microorganisms, mycorrhizal fungi play a crucial role in the formation and maintenance of soil aggregates and are considered one of the most important biotic factors influencing soil aggregation. A substantial contribution to soil aggregation and stability has been demonstrated for arbuscular mycorrhizae while the contribution of ectomycorrhizal fungi has not been comprehensively investigated yet (Graf and Frei, 2013). Arbuscular mycorrhizal fungi appear to be the most important mediators of soil aggregation for three reasons: (i) the extraradical hyphae of arbuscular mycorrhizal fungi represent a substantial component of soil microbial biomass, making up to 50% of fungal mycelia in soil (Gianinazzi et al., 2010); (ii) they are independent of the limiting carbon supply in bulk soil on which saprophytic fungi depend, by directly tapping into carbon resources of the plant; (iii) their hyphae appear to have a longer residence time in soil, allowing for a less transient contribution to soil-aggregate stabilisation than saprobic hyphae. Additionally, these fungi presumably act as a long-term soil-binding agent through the production of a stable putative glycoprotein, called glomalin. Glomalin is present in soils at high concentrations and is an important factor in stabilising aggregates and its concentration in aggregates (Wright and Upadhyaya, 1998) and soil (Rillig et al., 2001) correlates with the percentage of water-stable aggregates Rillig et al. (2002) reported a much stronger effect of glomalin on soil aggregation than the direct effect of arbuscular mycorrhizal fungi hyphae themselves, suggesting the high importance of the protein in hypha-mediated mechanism of soil aggregate stabilisation. Thus, the extensive networks of arbuscular mycorrhizal fungal hyphae play important roles in soil physical processes, particularly with regard to macroaggregates (> 250 um), while glomalin is tightly correlated with soil aggregate stability (Peng et al., 2013).

Hyphal networks of arbuscular mycorrhizae have an impact on the soil structure and plant-community composition and are therefore important below-ground carbon sinks (Soka and Ritchie, 2014). Wilson et al. (2009) observed that a loss of hyphal abundance of arbuscular mycorrhizae led to a concomitant cost in soil aggregation for which no other processes compensated. Similarly, increases in soil hyphae of arbuscular mycorrhizal fungi were correlated with an increased proportion of macroaggregates. Disturbance can affect the occurrence of arbuscular mycorrhizal fungi in both agricultural and natural ecosystems in several ways. First, it may change the physical, chemical or biological environment of soil leading to either direct effects on arbuscular mycorrhizal fungi or indirect effects operating via effects of disturbance on plant growth. Changes in symbiotic activity may be critical because a reduction in extramatrical mycorrhizal hyphae networks is likely to impact soil structure, soil C and N storage, and soil food webs. Wilson et al. (2009) showed a highly significant linear correlation between hyphal abundance of arbuscular mycorrhizal fungi and soil aggregation, and C and N sequestration with an experimental field study, involving long-term diverse management practices of native multispecies prairie communities. This suggested serious consequences to the loss of arbuscular mycorrhizal fungi from ecosystems.

By forming a complex intra- and extraradicular mycelial network arbuscular mycorrhizae confer a higher plant/soil adherence and contribute to soil stabilisation (e.g. Turrini and Giovannetti, 2012). In fact, together with the release of cementing agents, the ability to form an extra-radicular mycelium and the ability to render surfaces hydrophobic, are among the most important arbuscular mycorrhizal traits contributing to soil stabilisation (Rillig et al., 2015). Long-term field experiments revealed arbuscular mycorrhizal fungi abundance as the key factor explaining soil aggregation (Wilson et al., 2009).

In terms of the ecological entity to protect for this fungal group for this service, the risk assessment should be based on the functional group 'arbuscular mycorrhizal fungi'. This can be justified by the reasoning presented above for the other services. Moreover, Rillig et al. (2015) advocates the use of a trait-based approach to assess and understand the role of arbuscular mycorrhizae in soil stabilisation, which favours the adoption of 'functional group' as ecological entity to protect.

Table 8: Key drivers for the ecosystem service soil structure formation. Main taxa, examples of species and exposure routes.

Key drivers	Maintaxa	Main exposure routes	Example species
Macropores creators	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: *Lumbricus terrestris, Aporrectodea longa*
Soil mixers	Lumbricidae	Oral soil Contact soil	Endogeic worms *Aporrectodea caliginosa*
Litter/organic matter fragmenters	Oniscidae	Oral litter	Isopods: *Porcellio scaber*
	Lumbricidae	Oral soil Oral litter	Anecic worms: *Lumbricus terrestris, Aporrectodea longa*
	Collembola	Oral litter/org. matter Contact soil	*Folsomia quadrioculata, Pseudosinella alba, Entomobrya multifasciata, Lepidocyrtus cyaneus*
	Oribatida	Oral litter/org. matter Contact soil	Oribatid mites: *Oppiella nova, Tectocepheus velatus, Punctoribates punctum, Scheloribates laevigatus*
Aggregates stabilisers	Fungi and bacteria	Contact soil/contact soil pore water	
Glomalin producers	Arbuscular mycorrhizal fungi		

5.7. In-soil organisms as drivers of food web support

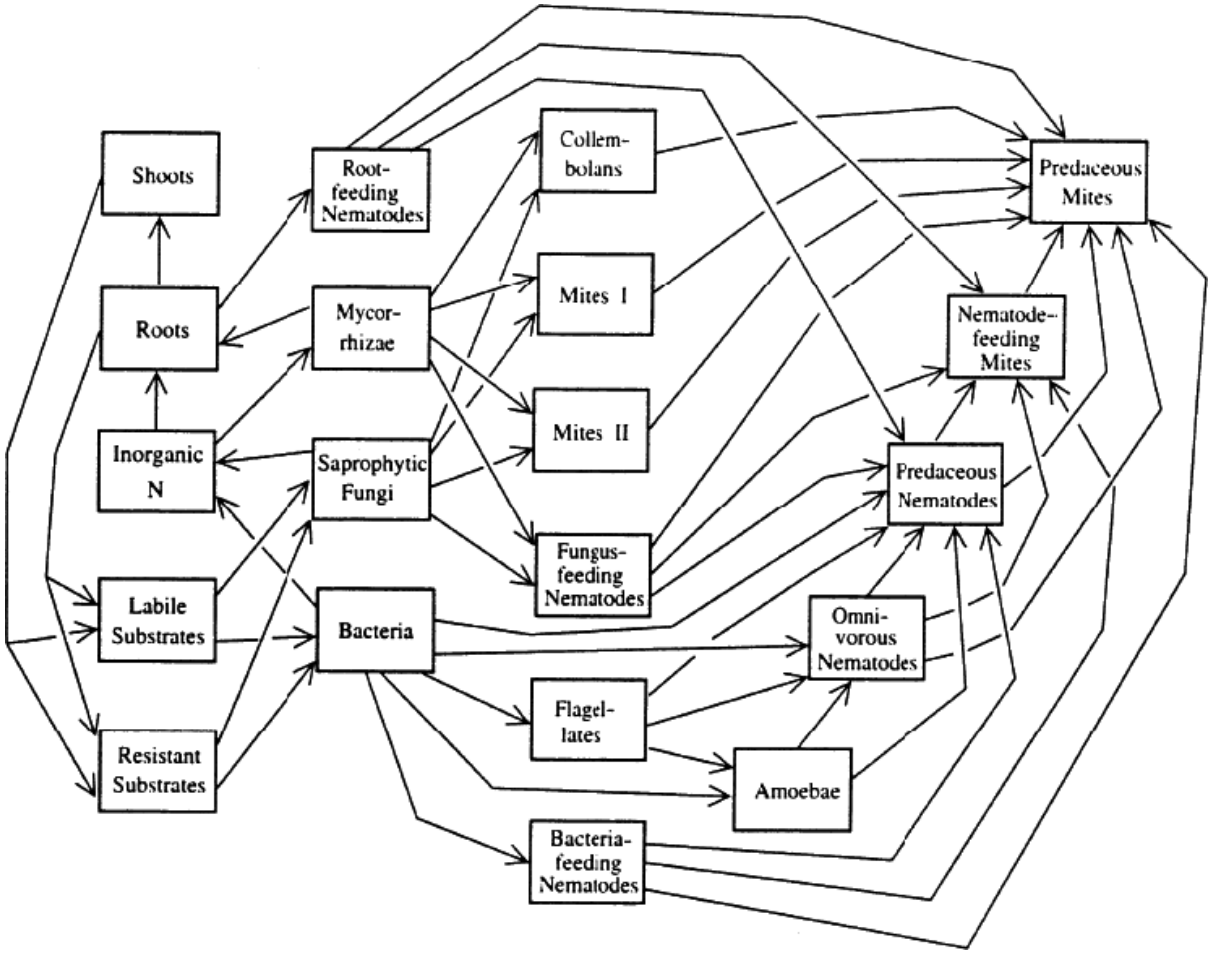

Figure 6: Soil food web (Reprinted from Hunt and Wall, 2002. Copyright John Wiley and Sons from Hunt et al., 1997)

Soil fauna

Although the overall importance of the in-soil invertebrate fauna to terrestrial food chains is rather poorly recognised, there are several species and food-chain links that have been relatively well studied. Most of them show unequivocally that at least certain in-soil invertebrate species are of crucial importance for supporting terrestrial food chains. Among the best recognised links between the soil environment and terrestrial fauna are those mammals and birds for which earthworms constitute an important food resource. The prime and long-known example is the Eurasian badger (*Meles meles*) with up to ca. 90% of food biomass consumed in the spring made up of earthworms (Goszczynski et al., 2000). The authors noted also an important difference between pristine forest habitats and farmlands. In forests, earthworms constituted on average 62% of the badger diet, while in farmlands and pastures earthworms and plant material were equally important (34% each). These differences are probably not without an effect on badgers: Kruuk and Parish (1985) found that badger body weight is positively correlated with earthworm consumption and suggested that long-term differences in earthworm availability affect badger population size. During their studies, the average badger biomass decreased gradually between 1978 and 1981, by 14% in males and 17% in females, and the trend was strongly correlated with the proportion of earthworms in the diet (see Figure 7). The authors hypothesised that the observed decrease in earthworm population size and availability to badgers resulted from changes in pasture management. Unfortunately, they did not study whether it was related to the use of any plant protection products. Nevertheless, these data clearly show the importance of earthworms for maintaining healthy populations of badgers.

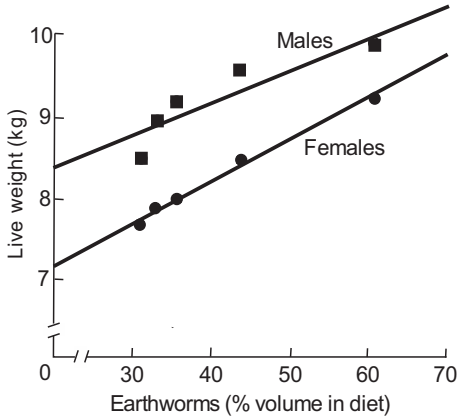

Figure 7: Relation between relative volume of earthworms in badger diet and the live weight of badgers during March-June: males: $r = 0.89$, $P < 0.05$; females: $r = 0.99$, $P < 0.001$. Redrawn after Kruuk and Parish (1985), copyright John Wiley and Sons

Earthworm availability was also the main factor besides altitude determining the spatial distribution of Eurasian Woodcock (*Scolopax rusticola*) at the landscape scale in Cantabrian Mountains in Spain (Brana et al., 2013). Indeed, according to Hoodless and Hirons (2007), earthworms can provide 50–80% of food biomass for this species.

While earthworms represent an important food resource for birds and mammals, many smaller in-soil invertebrates support a number of ground dwelling and aboveground invertebrate predators. For example, *Pardosa* spiders, which predate on both above-ground (e.g. Diptera) and small soil invertebrates, seem to prefer aphids when they are abundant but springtails prevail at low aphid densities (Kuusk and Ekbom, 2010) (see Figure 8). The authors conclude that the abundance of springtails may help in maintaining spider populations and, in consequence, enhance spider predation on pests.

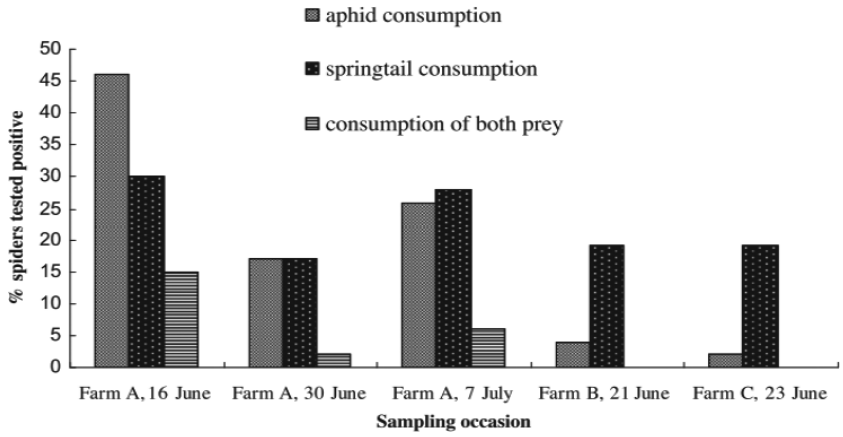

Figure 8: Percentage of *Pardosa* spiders collected in spring-sown cereals that tested positive for aphid consumption, springtail consumption or consumption of both prey by polymerase chain reaction (PCR)-based gut-content analysis. Reprinted from Kuusk and Ekbom (2010), Copyright (2010), with permission from Elsevier

Springtails are also the most numerous and important prey of epigeal linyphid spiders. Even if the spiders consume also substantial quantities of other prey, with aphids as the main component, Romero and Harwood (2010) noted that a diet consisting of aphids alone is not sufficient for spider development. Similar to Kuusk and Ekbom (2010), the authors stress the importance of spiders in controlling pest populations, especially aphids. At the same time, they found that spiders were severely prey limited and hypothesised that this is due to the impact of adverse climate conditions and agricultural practices on prey. If the latter is the case, the study by Romero and Harwood (2010) provides an important link between protection goals for in-soil invertebrates and non-target, above-ground arthropods (NTA). In the recently published 'Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods' (EFSA PPR Panel, 2015a), the crucial importance of landscape structure for the maintenance of NTA biodiversity has been stressed. The work by Romero and Harwood (2010) indicates that landscape structure may be of crucial importance in maintaining the important ecosystem function that small and less mobile soil invertebrates have in supporting higher trophic levels in a food chain.

A recent extensive study across four European countries, Sweden, the UK, the Czech Republic and Greece, showed that increasing land-use intensity negatively affects species richness of earthworms, springtails and oribatid mites and reduces mean body mass of soil fauna (Tsiafouli et al., 2015). A comparison of invertebrate prey abundance for birds between organic and conventional arable farms showed that earthworm abundance was 2–4 times higher on the former than on the latter (Kragten et al., 2011). Having in mind the trophic relationships described briefly above, this can also mean negative changes at higher trophic levels, including populations of important invertebrate and vertebrate carnivores.

Many birds, mammals, reptiles and insects are known to include gastropods in their diet, but there is a lack of studies about the ecological significance of malacophagy (Barker, 2004). As reviewed by Nyffeler and Symondson (2001), it has been shown for around 20 genera of spiders that they include gastropods in their diet. For harvestmen, gastropods are a particularly significant component in their diet, and some genera, mainly *Trogulus* and *Anelasmocephalus*, specialise in these prey. Their diet includes larger snails (e.g. family Helicidae) but also smaller sized species (e.g. families Vallonidae, Cochlicopidae, Pupillidae, Enidae, Endontonidae, see Nyffeler and Symondson (2001)). Carabid beetles of the tribe Cychrini, of which in Europe only the genus *Cychrus* occurs (Freude et al., 2005), principally feed on snails and slugs (Larochelle, 1972), and some species of the genus *Carabini* have specialised in eating snails and slugs (Freude et al., 2005).

Snail shells might be a vital calcium source for organisms at higher trophic levels. It has been shown for birds on poor soils in Dutch forests that a decline of snail densities resulted in eggshell defects in the great tit *Parus major* caused by calcium deficiency (Graveland and van der Wal, 1996). Graveland and Van Gijzen (1994) showed that forest passerines need to ingest calcium-rich items, e.g. snail shells, additionally to their normal food to satisfy their calcium requirements. It cannot be excluded that birds occurring in the agricultural landscape also require snails as a source of lime, but this topic requires further study.

Nematodes are important food sources for several species of other nematodes or soil microarthropods (Huhta et al., 1998), and opportunistic scavenging of dead invertebrates is common. Isotomid, entomobryid, tomocerid, and hypogastrurid collembolans have been found to correlate negatively with wood-decomposition rate, suggesting that direct feeding on fungi by these organisms may contribute to this negative relationship. Collembola form a significant proportion of the diets of ground-dwelling spiders, ground beetles (Carabidae), rove beetles (Staphylinidae), mesostigmatid mites, ants, diplurans, and other predacious arthropods (McBrayer and Reichle, 1971; Hopkin, 1997). Data on their importance as food supply for vertebrates are not available but, due to the small size of nematodes, they are probably negligible as direct food for larger animals.

The positions and roles of soil animals in the terrestrial food webs are extremely diverse. The ecological entity to be protected should be attributed therefore to functional groups in terms of position in the food web, but the functional groups are yet to be defined. From the point of view of present knowledge on food-web relationships, as described above, the following groups may be identified as crucial prey for different groups of animals: annelids, nematodes, gastropods, and arthropods. It should be also noted that the food-web relationships may be complex and multi-level, so that specific functional/taxonomic groups serve as food to other in-soil invertebrates which, in turn, are preyed upon by other animals, representing domains other than soil.

Soil microorganisms

Biotic interactions within soil food webs can be both bottom-up and top-down. Bottom-up interactions can be based on, for example, fresh root material for plant feeders, dead roots, root exudates and litter in the case of primary decomposers or prey in the case of secondary decomposers and predators. Top-down effects are mainly driven by predation (Turbé et al., 2010). Top-down regulation at higher trophic levels may appear marginal, considering that microbial biomass comprises the majority of the total biomass in soil. However, bottom-up interactions can be mainly ascribed to the prokaryotes and fungi (Mulder et al., 2005).

Prokaryotes, fungi and protozoa are considered key players in the soil food web, being at the bottom of the detritus food web. Thus, they are mainly eaten by all the other in-soil organisms including nematodes and protozoans. For example, the diet of earthworms mainly consists of organic material in various stages of decay. Dead plant tissue comprises the bulk of the organic matter consumed, but living microorganisms, nematodes and other microfauna, mesofauna and their dead remains are also ingested. It has been reported that earthworms are usually food generalists with a preference for dark pigmented fungi (Dematiacea), which often comprise up to 60% of fungal isolates from soil and are generally of high food quality. In addition to fungi, protozoa and algae may constitute a significant part of the diet of earthworms.

Most oribatid mite species appear to feed preferentially on certain genera of dark pigmented fungi, such as Alternaria and Ulocladium, but the food spectrum varies among oribatid mite species. Oribatid mites have also shown feeding preference for ectomycorrhizal fungi (Schneider et al., 2005). It has been reported, however, that some species can also be bacteria feeders, detritivorous species and some of them feed on carrion (Behan-Pelletier, 1999).

Nematodes can be classified according to their feeding behaviour in either bacterial, fungal or protozoan feeders. Like nematodes, protozoans can also be classified according to their feeding behaviour in bacterial or fungal feeders. Collembola have also been considered as generalist in their feeding behaviour feeding both on fungi and bacteria.

As presented above, soil microorganisms make up a taxonomically diverse yet single functional group in terms of food-web support. Even if some organisms show preferential feeding on certain groups of microorganisms, none is fully selective and generalists prevail. Hence, for SPGs, soil microorganisms should be regarded as one functional group, indispensable to support viable populations of a range of in-soil organisms.

Table 9: Key drivers for the ecosystem service food web support. Main taxa, examples of species and exposure routes.

Key drivers	Examplesof taxa	Main exposure routes	Example species
Soil non-arthropod invertebrates	Lumbricidae	Oral soil Oral litter Contact soil	Anecic worms: *Lumbricus terrestris, Aporrectodea longa* Endogeic worms: *Aporrectodea caliginosa*

Key drivers	Examplesof taxa	Main exposure routes	Example species
Soil non-arthropod invertebrates –small	Enchytraeidae	Oral soil Contact soil	*Enchytraeus* sp., *Fridericia* sp.
Litter macroarthropods	Isopoda	Oral litter, Contact litter	
Litter microarthropods	Collembola	Oral litter/org. matter Contact soil	Collembolans: *Folsomia quadrioculata Pseudosinella alba, Entomobrya multifasciata*, *Lepidocyrtus cyaneus*
	Oribatida s	Oral litter/org. matter Contact soil	Oribatid mites: *Oppiella nova, Tectocepheus velatus, Punctoribates punctum, Scheloribates laevigatus*
Microfauna	Nematoda	Contact soil	
Decomposers	Bacteria		
	Fungi including mycorrhizal fungi		
Grazers	Protozoan		

6. Specific Protection Goal Options for in-soil organisms in agricultural landscapes

6.1. Spatial scale in the environmental risk assessment for in-soil organisms

The spatial scale considered for in soil environmental risk assessment (ERA) cannot be divorced from a temporal scale. Given the slow rate of movement of many in-soil organisms, external recovery (by immigration) is slow and therefore consideration of long-term effects requires a large spatial scale covering multiple habitats. However, space may be important at small scales too since internal recovery via dispersal from more suitable refugia (microhabitats not affected by the stressor) towards impacted areas after the reduction in the stressor can occur. At the local in-field scale, the landscape may be described by a scale at which heterogeneity of one or more factors important for determining the population health of the organisms being assessed is considered (e.g. moisture, soil type, soil compaction, toxic chemicals). Between these two scales in both space and time we may need to consider boundary conditions between in-field and off-field. In the case of boundary scale, there is no suitable laboratory test and recourse would need to be made to modelling. As a result, we need three definitions of spatial scale with appropriate time scales:

1) Field scale (in- and off-field) – a local scale expressing heterogeneity of one or more habitat factors at a scale that determines local differentiation in population densities. Processes occurring over short to medium time scales of less than one season.
    a) **In-field:** piece of land for cultivation with crops, managed typically by one farmer.
    b) **Off-field:** area surrounding a field; either (semi)natural habitats with high ecological value (such as hedgerow or grass strip) or simple structures (fence or a bare strip of land); normally no short-term changes in cultivation, in most cases not to be influenced by the farmer. Another off-field category comprises man-made structures, e.g. an adjacent field, roads, etc.
    c) **In-crop:** the area actually cropped
    d) **Off-crop:** any uncropped area. It includes also uncropped areas in-field, and such areas can be, for example, the minimal required zone for agricultural management, buffer strips or beetle banks
2) Field-boundary scale – expressing variation between in-field and off-field habitat conditions and a time scale representing external recovery from off-field habitats to in-field to occur. Processes occur over a timescale of more than one season.
3) Landscape scale – representing habitat diversity at regional scales and capturing the spatiotemporal dynamics of in-soil organisms as they are affected by management and colonise or recolonise areas over long time periods. Processes occurring over a timescale of many years.

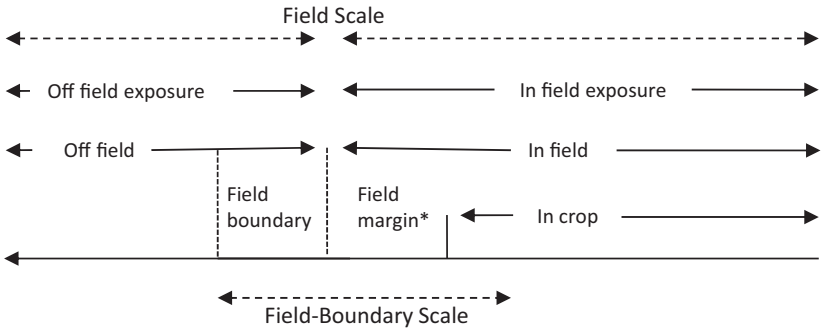

Figure 9: Spatial scales considered for the environmental risk assessment of in-soil organisms. Please, note that the landscape scale is not considered relevant for in-soil organisms. * If present as non-cropped or unsprayed area

Solid data on effects of landscape structure on in-soil organisms are next to non-existent, yet some conclusions about its importance for species richness can be drawn from the few studies on organisms that at least partly can be considered 'in-soil organisms'. One such group is the ground beetles (Carabidae) in which at least three stages (eggs, larvae and pupae) live in soil. This is also one of the most widely studied groups of terrestrial invertebrates, offering some insight into factors determining their abundance and diversity in different environments. For example, Trichard et al. (2013) showed that landscape structure explained four times more of the total variation in the abundance of granivorous carabids than local agricultural practices. Probably the most extensively studied group among all soil invertebrates is earthworms and there are some, albeit rather limited, data on landscape effects on species richness and biomass of this group. Vanbergen et al. (2007) showed that earthworm abundance and species richness were positively correlated with landscape-scale habitat richness, and Hof and Bright (2010) found that the presence of grassy field margins increases the abundance of earthworms (and carabids). Similarly, in the study by Nuutinen et al. (2011), field margins appeared crucial for colonisation of cultivated fields by the earthworm *Lumbricus terrestris*. The authors stressed the general importance of field margins in the dispersal ecology of earthworms in arable landscapes in the long term. It has to be mentioned, however, that at least one study showed that under certain circumstances the earthworm biomass is actually higher in cultivated land than in small non-cultivated areas in Northern Europe. Lagerlof et al. (2002) found that when soil cultivation is moderate and organic matter is added, large populations of most earthworm species can be found in cultivated fields. Nevertheless, they also found that some epigeic species, such as *Lumbricus rubellus* and *L. castaneus*, are more abundant in non-cultivated areas from where they can migrate to the fields in the long term. The authors stressed that their findings are restricted to fields with moderate cultivation intensity and that, if heavy cultivation is used with soil compaction and intensive pesticide use, non-cultivated areas are crucial for preserving earthworm populations in agricultural landscapes.

Yet another aspect worth taking into account in landscape-level risk assessment is the importance of soil invertebrates for terrestrial food chains. Kauhala and Ihalainen (2014) proved that the higher the landscape diversity the higher was the diversity of diet of the badger (*Meles meles*) and the raccoon dog (*Nyctereutes procyonoides*). It is generally accepted that high diet diversity is beneficial for animals, so the study indirectly shows that maintaining diverse landscapes promotes healthy populations of mammal omnivores by securing a diverse diet. For more information on food-chain effects, see Section 5.7 of this opinion.

Considering the time-scales and biological processes related to the dispersal of the majority of in-soil organisms compared to terrestrial non-target arthropods living above soil, the Panel proposes that in-soil environmental risk assessments are made at local scales, considering processes at the field boundary level. For NTAs, the primary justification for making assessments at a landscape scale was the need to take 'action at a distance' into account (see EFSA PPR Panel, 2015a; Topping et al., 2015a). This effect will occur when spatial-dynamic processes operate over a timescale whereby depletion of source populations can occur. In the case of most in-soil organisms, the rate of internal recovery (driven by reproduction) is likely to be much higher than any change in local density driven by dispersal (see Sections 3.2.1 and 3.2.2). As a result, 'action at a distance' is not expected to be important for most in-soil organisms, and thus landscape-level assessment is not needed. In addition, timescales for landscape-level processes are long since the dynamics considered at that scale are

related to recovery following long-term agricultural use of soils. However, assessment of year on year effects on in-soil organisms is considered important, since small effects from exposure to PPPs in the field within one season might accumulate over multiple years of PPPs use (Liess et al., 2013).

6.2. SPG Options for in-soil organisms as service providing units

The proposed different options for Specific Protection Goals for in-soil organisms in agricultural landscape are derived by combining the knowledge summarised in the sections above regarding the determined key drivers with their pertinent ecologically entities (Service Providing Units or SPU according to EFSA Scientific Committee, 2016b) and the traits of the SPU in terms of their recovery and dispersal potential (see Sections 3.2.1 and 3.2.2 above). These data are integrated to derive a magnitude of effects by intended PPP use that might be acceptable without compromising the delivery of the ecosystem services of interest.

In the trade-off between crop production and protection of biodiversity and ecosystem services, the Panel might propose a higher level of effects on diversity in-field than off-field. In doing this, the Panel acknowledge that in-field crop protection might be 'rated' higher in term of provisioning service than biodiversity and other ecosystem services. This is not the case for off-field SPG Options.

Therefore, the proposed SPG options for the in-field areas are given as limits of operation of the addressed service providing unit, i.e. the maximum tolerable magnitude of effects on the key drivers and their entities in order to be (still) able to deliver the identified ecosystem service. If lower magnitude of effects than the limits of operation is considered as pertinent for the in-field area by risk managers (e.g. negligible effects), then no consequences for the service provision are expected. If higher magnitude of effects are considered relevant, then consequences regarding the ecosystem service provision and the long-term persistence of the populations are to be expected. The consequences of choosing different SPG Options are explained in Section 6.3. For reason of simplicity, the proposed SPG Options are given as 'Option: Below the limit of operation', 'Option: Limit of operation' and 'Option: Above limit of operation' for the service providing units.

As discussed in Section 5, species diversity plays a key role for the long-term performance of functional groups in strongly disturbed agricultural soils. When identifying the 'functional group' as SPU for the maintenance of certain ecosystem services, it should be kept in mind that the definition of SPU solely at the level of functional groups might lead under unfavourable conditions to a loss of function performance. Species loss within a functional group will lead to the erosion of trait diversity and to a reduced resilience and stability under changing environmental conditions. In order to support the long-term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended to define the SPU as the abundance/biomass of the populations of species belonging to the different functional groups. For the off-field non-target areas, it is proposed that only negligible effects on the abundance/biomass of in-soil organisms' populations can be tolerated.

In terms of this Opinion, the definition of possible acceptable magnitude of effects as percentage reduction compared to a 'control' applies to a defined context. For example, in an agricultural system supporting a high diversity of in-soil organisms, a given reduction (e.g. 50%) may still retain the function represented by the SPG. In contrast, in landscapes with very low in-soil diversity, the acceptability of effects might be at a far lower magnitude level, e.g. removing 50% of 2 species may be critical. This context dependency applies to all proposed SPG options for in-soil organisms.

For in-field as well as off-field areas, the tolerable magnitude of effects should take multiple PPP applications according to typical PPP 'spray schedules' into account. This will possibly implicate a lower level of tolerable effects for single PPP applications, especially in-field, if the intended use fits in an application scheme that includes several other PPPs with potential effects on in-soil organisms in the crop. Multiple applications of several PPPs in typical schedules should also be taken in consideration when addressing the recovery of in-soil organisms.

The proposed tolerable magnitudes of effects are related to the protection goals and not to the detectable limits of measurement endpoints. Although, the Panel acknowledges that the proposed tolerable magnitude of effects, from 10% to < 35%, might not be possible to detect for some endpoints under certain circumstances, it has been proven that with proper experimental design, effects within the proposed range can be detectable (see Section 9.7.2).

6.2.1. SPG Options for Earthworms

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be small effects less than 35% up to months on the ecological

entity 'populations of different earthworm species'. Please refer to Section 4 for the definition of effect size and temporal scales of effects. This magnitude of effect is deemed to allow for internal recovery of earthworm populations so that biodiversity levels and the provision of the ecosystem-services in agricultural field soils is assured in relevant time frames (please refer to Section 3.2). For earthworm populations, medium effects higher than 65% would not result in internal recovery in relevant time frames. Table 10 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 10: Specific Protection Goal Options for Earthworms

Earthworms			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population	Abundance/biomass	Small effects up to months
Nutrient cycling	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months
Pest control	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months
Natural attenuation	Minor importance		
Soil structure	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months
Food web support	Functional groups	Abundance/biomass	Small effects up to months
Off field			
Biodiversity and all ecosystem services	Population	Abundance/biomass	Negligible effects/temporal scale not relevant

6.2.2. SPG Options for Enchytraeids

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be small effects less than 35% for months on the ecological entity 'populations of different enchytraeid species' or medium effects less than 65% for weeks. These magnitudes of effects are deemed to allow for internal recovery of enchytraeids populations (see Section 3.2), so that biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in relevant time frames. Table 11 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 11: Enchytraeids

Enchytraeids			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Nutrient cycling	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks

Enchytraeids			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
Pest control	Minor importance		
Natural attenuation	Minor importance		
Soil structure	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Food web support	Functional groups	Abundance/biomass	Small effects up to months Medium effects up to weeks
Off field			
Biodiversity and all ecosystem services	Population	Abundance/biomass	Negligible effects/temporal scale not relevant

6.2.3. SPG Options for Microarthropods

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be medium effects less than 65% for weeks on the ecological entity 'populations of different microarthropod species' or small effects less than 35% for months. These magnitudes of effects are deemed to allow for internal recovery of microarthropod populations, so that biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in relevant time frames (see Section 3.2). Table 12 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 12: Specific Protection Goal Options for Microarthropods

Microarthropods			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Nutrient cycling	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Pest control	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Natural attenuation	Minor importance		
Soil structure	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Food web support	Functional groups	Abundance/biomass	Small effects up to months Medium effects up to weeks
Off field			
Biodiversity and all ecosystem services	Population	Abundance/biomass	Negligible effects/temporal scale not relevant

6.2.4. SPG Options for Macroarthropods (e.g. Isopods)

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal are suggested to be medium effects less than 65% for weeks on the ecological entity 'populations of different macroarthropod species' or small effects less than 35% for months. These magnitude of effects will allow for internal recovery and recolonisation by macroarthopod species (see Section 3.2), so that biodiversity levels and the provision of ecosystem services in agricultural field soils is assured in relevant time frames. Table 13 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and

the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 13: Specific Protection Goal Options for Macroarthropods

Macroarthropods (e.g. Isopods)			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Nutrient cycling	Long-term persistence of functional groups → population	Abundance/biomass	Small effects up to months Medium effects up to weeks
Pest control	Minor importance		
Natural attenuation	Minor importance		
Soil structure	Minor importance		
Food web support	Functional groups	Abundance/biomass	Small effects up to months Medium effects up to weeks
Off field			
Biodiversity and all ecosystem services	Population	Abundance/biomass	Negligible effects/temporal scale not relevant

6.2.5. SPG Options for terrestrial Gastropods (slugs and snails)

Small effects < 35% for months on the ecological entity population of different gastropod species are suggested as the maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal. For gastropod species, the choice of medium effects over period of weeks does not appear suitable to enable internal recovery in relevant time frames (see Section 3.2.1). Table 14 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 14: Specific Protection Goal Options for terrestrial gastropods

Terrestrial gastropods (slugs and snails)			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population	Abundance	Small effects up to months
Nutrient cycling	Long-term persistence of functional groups → population	Abundance	Small effects up to months
Pest control	Minor importance		
Natural attenuation	Minor importance		
Soil structure	Long-term persistence of functional groups◦population	Abundance	Small effects up to months
Food web support	Functional groups	Abundance	Small effects up to months
Off field			
Biodiversity and all ecosystem services	Population	Abundance	Negligible effects/temporal scale not relevant

6.2.6. SPG Options for Nematodes

The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal are suggested to be medium effects less than 65% for weeks or small effects less than 35% for months. Regarding the maintenance of biodiversity levels close to normal operating ranges for agricultural field soils in relevant time frames, the ecological entity to be assessed should be 'community structure of nematodes'. In addition, the allocation of nematodes to trophic groups helps to condense information efficiently and to determine their contribution to ecosystem services. Table 15 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 15: Specific Protection Goal Options for Nematodes

Nematodes			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Population/community	Abundance/community structure	Small effects up to months Medium effects up to weeks
Nutrient cycling	Functional group	Abundance	Small effects up to months Medium effects up to weeks
Pest control	Functional group	Abundance	Small effects up to months Medium effects up to weeks
Natural attenuation	Minor importance		
Soil structure	Minor importance		
Food web support	Functional group	Abundance	Small effects up to months Medium effects up to weeks
Off field			
Biodiversity and all ecosystem services	Population/community	Abundance/community structure	Negligible effects/temporal scale not relevant

6.2.7. SPG Options for Mycorrhiza, other fungi and protozoa

Fungi have a short generation time and good dispersal ability (see Section 5), which allows them to recover quite fast. The maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is medium effects (35 < 65%) up to weeks on the ecological entity population or functional group of different fungi, including mycorrhizal fungi and protozoan species, depending on the ecosystem service. For the maintenance of biodiversity levels close to normal operating ranges for agricultural field soils in relevant time frames, the ecological entity to be assessed should be the 'community' and the attribute structure (phylogenetic or functional). The proposed magnitude of effect is deemed to allow for internal recovery (see Section 3.2) so that biodiversity levels and the provision of the ecosystem service genetic resource in agricultural field soils is assured in relevant time frames. Table 16 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 16: Specific Protection Goal Options for Mycorrhiza, other fungi and protozoa

Mycorrhiza, other fungi and protozoa			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Community	Structure	Small effects up to months Medium effects up to weeks
Nutrient cycling	Functional group	Abundance/biomass	Small effects up to months Medium effects up to weeks
Pest control	Functional group	Abundance/biomass	Small effects up to months Medium effects up to weeks
Natural attenuation	Functional group	Abundance/biomass	Small effects up to months Medium effects up to weeks
Soil structure	Functional group	Abundance/biomass	Small effects up to months Medium effects up to weeks
Food web support	Functional group	Abundance/biomass	Small effects up to months Medium effects up to weeks
Off-field			
Biodiversity and all ecosystem services	Community	Structure	Negligible effects/temporal scale not relevant

6.2.8. SPG Options for Soil Bacteria and Archaea

Soil bacterial and archaean communities may recover from disturbance quite fast, mainly because their generation time is short, their rates of passive dispersal and recolonisation are comparatively high (see Section 3.2), and because of functional redundancy. For services connected with genetic resources and biodiversity, it is suggested that the ecological entity should be microbial community and the attribute diversity (phylogenetic or functional). For other ES, the proposed ecological entity to be protected is functional group. It is suggested that the maximum magnitudes of effect that might be tolerated in-field are large effects up to days, medium effects up to weeks or small effects up to months. Table 17 gives for the in-field area the limits of operation of the service providing unit, so that the respective services can (still) be delivered and the long-term persistence of the populations is assured. Lower magnitude of effects might be chosen also for the in-field area (e.g. negligible effects), resulting in no expected effects on the service provision. If higher magnitude of effects is chosen, then consequences for the service provision and for the service providing unit are expected (see for this Section 6.3).

Table 17: Specific Protection Goal Options for Soil Bacteria and Archaea

Soil Bacteria and Archaea			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field			
Biodiversity, genetic resources, cultural services	Microbial community	Diversity	Small effects up to months Medium effects up to weeks Large effects up to days
Nutrient cycling	Functional group	Abundance/ biomass/activity	Small effects up to months Medium effects up to weeks Large effects up to days
Pest control	Functional group	Abundance/ biomass/activity	Small effects up to months Medium effects up to weeks large effects up to days
Natural attenuation	Functional group	Abundance/ biomass/activity	Small effects up to months Medium effects up to weeks Large effects up to days
Soil structure	Minor importance	Abundance/ biomass/activity	Small effects up to months Medium effects up to weeks Large effects up to days

Soil Bacteria and Archaea			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
Food web support	Functional group	Abundance/ biomass/activity	Small effects up to monthsMedium effects up to weeks Large effects up to days
Off field			
Biodiversity and all ecosystem services	Microbial community	Diversity	Negligible effects/temporal scale not relevant

6.3. Consequences of choosing different Specific Protection Goal Options for in-soil organisms key drivers

Limits of operation for the key drivers are given in Section 6.2 (Tables 10–17) and Section 4. The proposed SPG options (especially for the in-field areas) are given as limits of operation of the addressed service providing unit so than it can (still) deliver the identified ecosystem service. If lower magnitude of effects as the limits of operation is considered by risk managers (e.g. negligible effects also in the in-field areas), then no consequences for the service provision are expected. If magnitude of effects higher that the ones reported in tables 10 to 17 are considered relevant, then unacceptable consequences regarding the ecosystem service provision and the long-term persistence of the populations are to be expected. An overview table (Table 18) with the proposed in-field SPG options is presented below. The consequences of choosing different SPG Options are explained in the Table 19.

Table 18: Overview of the proposed in-field protection goal options

Organism group	Ecological entity/attribute	Option: below the limit of operation	Option: limit of operation	Option: above the limit of operation
		Magnitude and Duration	Magnitude and Duration	Magnitude and Duration
Earthworms	Population/abundance – biomass	Negligible effects Small effect up to weeks	Small effect up to months	Medium effects for months
Enchytraeids	Population/abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for months
Microarthopods	Population/abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Macroarthropods	Population/abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Gastropods	Population/abundance – biomass	Negligible effects Small effect up to weeks	Small effect up to months	Medium effects for month
Nematodes	Population/abundance – biomass	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Mycorrhiza, other fungi and protozoa	Community/structure	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks	Medium effects for month
Soil bacteria and Archaea	Community/microbial community	Negligible effects Small effects up to weeks Medium effects up to days	Small effect up to months Medium effects up to weeks Large effects up to days	Medium effects for months Large effects for weeks

Table 19: Consequences of option choice regarding the effects of intended PPP use on in-soil organisms

	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Below limit of operation	**Option: Limit of operation**	**Option: Above limit of operation**
Biodiversity, genetic resources, cultural services (all in-soil organism groups in the scope of this Opinion)	The upper level of the normal operating range for in-soil organism communities in agricultural landscapes is sustained. Species-specific interactions, food-web structure and ecosystem processes are unaffected by the intended PPP use. General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in Regulation (EC) No. 1107/2009[(a)] is fully achieved. Support of the target 'Increase the contribution of agriculture to maintaining and enhancing biodiversity' (3a) of the EU 2020 Biodiversity Strategy[(a)], which has shown no significant progress so far. This Option contributes to Action 10 of the EU 2020 Biodiversity Strategy[(a)]: 'The Commission and Member States will encourage the uptake of agri-environmental measures to support genetic diversity in agriculture and explore the scope for developing a strategy for the conservation of genetic diversity'. The aims of Council Directive 92/43/EEC[(b)] on the conservation of natural habitats and of wild fauna and flora are achieved	The limit of operation identified in the SPG tables marks a tipping point for the normal operating range of in-soil key drivers delivering genetic resources and cultural services and supporting all ecosystem services. Reduction in species diversity reduces the efficiency with which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients. Biodiversity is supported to a degree that insures the long-term functioning of agricultural system, even if sensitive species are affected in the short term and species-specific interactions might be disrupted. General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in Regulation (EC) No. 1107/2009 is still achieved if off-field areas of pertinent size in a diversified landscape sustain the upper level of biodiversity normal operating range	Species loss above a tipping point may force ecosystems to move to a different (locally) stable state or to collapse. Loss of biodiversity will weaken the ability of agricultural ecosystems to respond to external changes such as climate change (loss of stability and resilience). Biodiversity losses will lead to disruption of valuable ecosystem functions thereby reducing delivered services. Cultural services will be reduced if vulnerable species decline or disappear. General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in Regulation (EC) No. 1107/2009 is not achieved. The target 'Increase the contribution of agriculture to maintaining and enhancing biodiversity' (3a) of the EU 2020 Biodiversity Strategy[(a)] will most probably not be met. The aim of halting of biodiversity loss by 2020 is not achieved: 'Halting biodiversity loss constitutes the absolute minimum level of ambition to be realised by 2020' (2009/2108(INI)[(c)] and 2011/2307(INI)[(d)]. UN sustainable development goals (SDG)[(e)] Sustainable Goals 2.4 and 15.5 are jeopardised. These goals are: 'By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality' and 'Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species'

	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Below limit of operation	**Option: Limit of operation**	**Option: Above limit of operation**
Nutrient cycling (in particular: litter fragmenters, soil organic matter feeders, bacteria and fungi feeders, mineraliser, see Table 4)	Upper limit of the normal operating range of soil organisms as drivers of organic matter decomposition and nutrient cycling is supported. The aims of the EU thematic strategy for soil protection[(f)] to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' are fully supported. UN sustainable development goals (SDG) 14 Sustainable Goals and 2.4 and 12.2 are supported These goals are: 'By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality' and 'By 2030, achieve the sustainable management and efficient use of natural resources'	This limit of operation marks the lower threshold of the normal operating range for soil organisms in the decomposition of dead organic matter and the delivering of nutrients.The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009 and the goal of the EU thematic strategy for soil protection to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' are still met in the long term. Nutrient availability and plant productivity are not impaired in the long term, even if vulnerable species in functional groups might be affected in the short term. To ensure this, off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term.	Reduced nutrient availability might reduce plant productivity. The requirements for external inputs of nutrients to maintain crop yield will increase. Disruption of trophic networks can occur, impairing the ecological equilibrium of the system.The aims of the EU thematic strategy for soil protection[(d)] to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' may not be met. UN sustainable development goals (SDG)[(c)] 2.4 and 12.2 are jeopardised. These goals are: 'By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality' and 'By 2030, achieve the sustainable management and efficient use of natural resources'

	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Below limit of operation	**Option: Limit of operation**	**Option: Above limit of operation**
Pest and pathogen control (in particular: pest pathogen competitors and suppressors, toxin dispersal antagonists, see Table 5)	Control of specific pest and pathogens by soil organisms is at the upper level of the normal operating range for agricultural soils. Aims of Directive 2009/128/(g) for achieving a sustainable use of pesticides are fully supported: 'Member States shall establish or support the establishment of necessary conditions for the implementation of integrated pest management. In protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures'	Resilient organisms will still deliver the service of pest and pathogen control in agricultural soils. However, control of specific pathogens by vulnerable soil organism key drivers might be reduced in the short term. The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009 and the aims of Directive 2009/128/(e) for achieving a sustainable use of pesticides are still implemented, as long as off-field areas of pertinent size in a diversified landscape should deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term	Enhanced proliferation of pest and pathogens through the disruption of intra- and interspecies interaction within the soil community (competition, predation, and parasitism) might finally lead to reduced plant productivity. Pests and pathogens may increase both numerically and in geographical spread, leading to greater reliance on chemical pesticides and further reduction in biodiversity. Aims of Directive 2009/128/(e) for achieving a sustainable use of pesticides are not implemented: 'Member States shall establish or support the establishment of necessary conditions for the implementation of integrated pest management. In protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures'
Natural attenuation (in particular: microorganisms and soil fauna influencing the biodegradation, dispersion, sorption and; mineralisation of contaminants, see Table 6)	The aims of the EU thematic strategy for soil protection(d) to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' are fully supported. UN sustainable development goals (SDG) 6.3 and 15.3 are supported. These goals are: 'By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally' and 'By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and	The upper limit of the normal operating range for soil organisms to perform natural attenuation of contaminants in agricultural soils are reached. The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009 and the aims of the Water Framework Directive (WFD)(h) that commits European member states to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater is still supported, even if the degradation of specific compounds by specialised vulnerable soil organisms might be hampered in the short term	Slower removal and attenuation of contaminants from soil. Reduction in soil fertility through microbial primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of soil. Potential leaching of contaminants to groundwater and run-off/drainage entry into surface water. The aims of the Water Framework Directive (WFD)(f) that commits European member states to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater may not be fulfilled. The aims of the EU thematic strategy for soil protection to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' may not be met. UN sustainable development goals (SDG) 6.3 and 15.3 are jeopardized. These goals are: 'By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally' and

	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Below limit of operation	**Option: Limit of operation**	**Option: Above limit of operation**
	floods, and strive to achieve a land degradation-neutral world'		'By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world
Soil structure formation and water retention (in particular: so-called soil ecosystem engineers, macropores creators, soil mixers, litter and organic matter fragmenters, aggregates stabilisers and glomalin producers, see Table 8)	Soil aggregate stability is increased, organic matter is incorporated into the soil profile and soil profile development is supported. Stabilisation of organic matter and carbon sequestration in soil aggregates will protect carbon-rich detritus from microbial degradation. Soil structure formation and support will prevent water logging, oxygen depletion and increased denitrification Movement of soil organisms in the soil profile will reduce hydrophobic patches formation. The aims of the EU thematic strategy for soil protection to 'protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms' is fully supported. UN sustainable development goal (SDG) 2.4 is jeopardised. This goals states: 'By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality'	In-soil key drivers of soil structure formation and water retention will deliver these services at the lower limit of the normal operating range. Vulnerable key drivers might be affected by PPP use in the short term.The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009 and the aims of EU thematic strategy for soil protection[(d)] are still implemented, as long as off-field areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms in the middle and long term	Soil structure disruption may lead to soil compaction in vulnerable soils, which is not broken up by soil organism key drivers. Less macropores will increase water logging in vulnerable soils, less connecting micropores to lower water holding capacity.Increased surface run-off and erosion may lead to contaminant and nutrient entries into surface waters. Risk of floods increases. The aims of the Water Framework Directive (WFD)[(f)] to achieve a good ecological and chemical status for surface waters and a good quantitative and chemical status for groundwater may not be fulfilled. The aims of Council directive 91/676/EEC[(i)] Concerning the protection of waters against pollution caused by nitrates from agricultural sources may not be fulfilled. Agricultural areas may be classified as 'areas facing natural constraints' according Regulation (EU) No 1305/2013[(j)], which might cause additional costs to the European Community in the form of payments to farmers to maintain agriculture in these areas. To receive direct payments in the context of the EU Common Agricultural Policy (CAP), farmers shall maintain land in good agricultural and environmental condition. Soil degradation may lead to increased effort to maintain the reference conditions or even to the loss of direct payments. UN sustainable development goal (SDG) 2.4 is jeopardised. This goals states: 'By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality'

	Consequences of option choice regarding the effects of intended PPP use on in-soil organisms		
	Option: Below limit of operation	**Option: Limit of operation**	**Option: Above limit of operation**
Food web support (all in-soil organisms as part of the soil food web and as food provision for species at higher trophic level, see Table 9)	Structure and functioning of the soil food web in agricultural soils is preserved and the support of all above-ground terrestrial food webs is achieved. Vulnerable species at higher trophic level, e.g. farmland birds, that are highly dependent on invertebrates for chick growth and survival will be supported. The aim of halting of biodiversity loss by 2020 is fully supported: 'Whereas the disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values' (e.g. 2009/2108(INI) and 2011/2307(INI)	Disruption of trophic networks can occur when vulnerable soil organisms are affected by PPP intended uses in the short term, impairing the ecological equilibrium of the system. The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009; the aims of Council Directive 79/409/EEC(k) on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are still achieved in the long term – as long as off-field areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery and recolonisation of vulnerable soil organisms	Vulnerable species at higher trophic level, e.g. farmland birds, that are highly dependent on invertebrates for chick growth and survival, will decline further and may become extinct. Diverse income-earning activities such as game-bird shooting may recede, leading to reduced financial viability of farms. General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in Regulation (EC) No. 1107/2009 is not achieved. Aims of Council Directive 79/409/EEC on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are not achieved. The aim of halting of biodiversity loss by 2020 is not achieved: 'Whereas the disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values' (e.g. 2009/2108(INI) and 2011/2307(INI))

(a): European Union: Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. 21 October 2009. Official Journal of the European Union L 309, 24 November 2009, 50 pp.
(b): Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.
(c): 2009/2108(INI) Report on the implementation of EU legislation aiming at the conservation of biodiversity.
(d): 2011/244(INI) Communication: on our life insurance, our natural capital: an EU biodiversity strategy to 2020 Committee on the Environment, Public Health and Food Safety.
(e): United Nations General Assembly (2015): Resolution adopted by the General Assembly on 25 September 2015. Transforming our world: the 2030 Agenda for Sustainable Development. Distr. General, 21 October 2015. Seventieth session, Agenda items 15 and 116, A/RES/70/1, 35 pp.
(f): COM/2006/0232 final (2006): Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC.
(g): Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides.
(h): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.
(i): Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources.
(j): Regulation (EU) No 1305/2013 of the European Parliament and of the Council of 17 December 2013 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD) and repealing Council Regulation (EC) No 1698/2005.
(k): Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds.

6.4. Option according to the current risk assessment scheme

Table 20: Option according to the current scheme (European Commission, 2002; EPPO, 2003)

Soil organisms and functions			
	Ecological entity	**Attribute**	**Magnitude/temporal scale**
In-field-option			
Earthworms	Population	Abundance/ biomass	Effects > 50% observed during a study, but with full recovery within 1 year
Soil microorganisms (mainly bacteria and fungi but not mycorrhizae)	Functional group	Process	< 25% after 28 days < 25% between 42 and 100 days

In the current scheme, a tiered approach is implemented only for earthworms and partially for microorganisms. For those groups of organisms, the tolerable magnitude and duration of effects is reported in the Table 20.

However, considering some biological features of earthworms, the magnitude of effect as proposed in the current system is not deemed to allow for internal recovery of earthworm populations. For microorganism, a tiered approach is not fully implemented. The litter bag study is recommended as the only higher tier functional test. However, this test is not specific for microorganisms being an integrated measurement of activity where redundancy can occur and litter biodegradation can be observed even if some species or functional group have been lost or their abundance has been highly reduced.

However, having only a protection goal for earthworms and partially for microorganisms does not assure protecting biodiversity and the provision of the ecosystem-services in agricultural field as defined in Section 5. For example, mycorrhizae are not currently specifically addressed in the risk assessment, while as explained in Section 5.6, they can provide services like soil formation that once impacted can require very long time before it can be recovered. It has also to be noted that while at Tier 1 toxicity studies with collembolan and mites are quite often required, there is neither a tolerable magnitude of effects nor an approach to refine the risk for those groups of organisms at structural level. Those groups are highly diverse and have a crucial role as key drivers of many of the relevant ecosystem services. Indirect effects are also not addressed in the current scheme.

In addition, in the current system only the in-field risk assessment is carried out, while it is proposed to assess the risk in both the in-field and off-field areas also considering processes at the field boundary since recovery via dispersal can occur over small scales.

6.5. Does persistence of plant protection products in soil need additional assessment?[11]

The SPGs as set up have a spatial and a temporal aspect, and they consider the agricultural context. The question is whether residues of PPPs can result in unexpected or unwanted effects on the long-term, even when the SPGs are met. From a point of view of sustainable land use, the use of PPPs should not hamper the use of soil for other functions in future, e.g. after agricultural land is taken out of production. As soon as the use or the function has changed, there is a possibility that residues of PPPs may not comply with the SPGs for the new situation – if this possible change in function was not addressed in the authorisation procedure. It is proposed during the development of the guidance document on the risk assessment for in-soil organisms to do some example calculations with relatively persistent PPPs in soil in order to find out whether it could be necessary to include an additional assessment for persistence of PPPs. In Appendix D, an example is given of a possible approach to include persistence into the risk assessment. Note that Appendix D is a summary of a proposal developed in 2006. When additional assessment of persistence is deemed necessary, triggers and methods have to be adjusted to present regulation and risk assessment methodology.

[11] The assessment of the persistence of substances in order to address the cut-off and the substitution criteria is not in the remit of this Opinion.

7. General Framework

This chapter presents the opinion of the PPR Panel on how in principle the environmental risk assessment (ERA) for in-soil organisms exposed to intended uses of Plant Protection Products (PPPs) should be conducted in the future, based on the best scientific knowledge available.

Besides some general words on the principles of tiered ERA schemes, this chapter will focus on the selection of the surrogate reference tier (SRT) and how to deal with the different level of uncertainty at the different tiers, including the calibration of lower tiers. A flowchart is presented with a proposal on how to conduct ERA. In addition, a section on the identification of the vulnerable species/groups and on how to link exposure and effects has also been included.

7.1. The principles of a tiered approach

The guidance document for aquatic risk assessment (EFSA PPR Panel, 2013) provides an overview of the principles of the tiered approach and the need to adopt them when assessing environmental risks of PPPs to optimise costs and increase the efficacy of the assessment. According to Boesten et al. (2007) and Solomon et al. (2008), the general principles of tiered approaches are:

- lower tiers are more conservative than higher tiers;
- higher tiers aim at being more realistic than lower tiers;
- lower tiers usually require less effort than higher tiers;
- in each tier, all available relevant scientific information is used;
- all tiers aim to assess the same protection goal.

Thus, the tiered system needs to be (i) appropriately protective, (ii) internally consistent, (iii) cost-effective, and (iv) it needs to address the problem with a higher accuracy and precision when going from lower to higher tiers (see Figure 10).

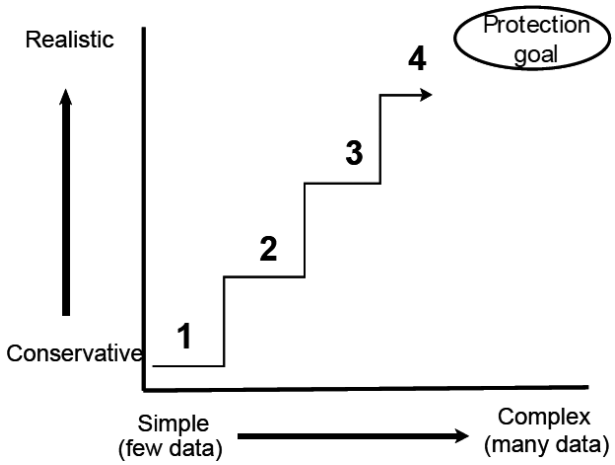

Figure 10: Tiers in the risk assessment process, showing the refinement of the process through the acquisition of additional data (EFSA PPR Panel, 2010a)

7.2. Tiered approach in the risk assessment for in-soil organisms and definition of (surrogate) reference tier

A tiered approach implies the existence of a surrogate reference tier (SRT), i.e. a representation, as accurate as possible, of the real situation in the field (i.e. the reference tier). This SRT should link the assessment being performed and the specific protection goals (see Figure 11). A SRT is a compromise between what would be desirable and what is practical. The SRT should be used to calibrate the lower tiers properly in order to make them sufficiently protective, taking into account the level of protection defined in the SPGs.

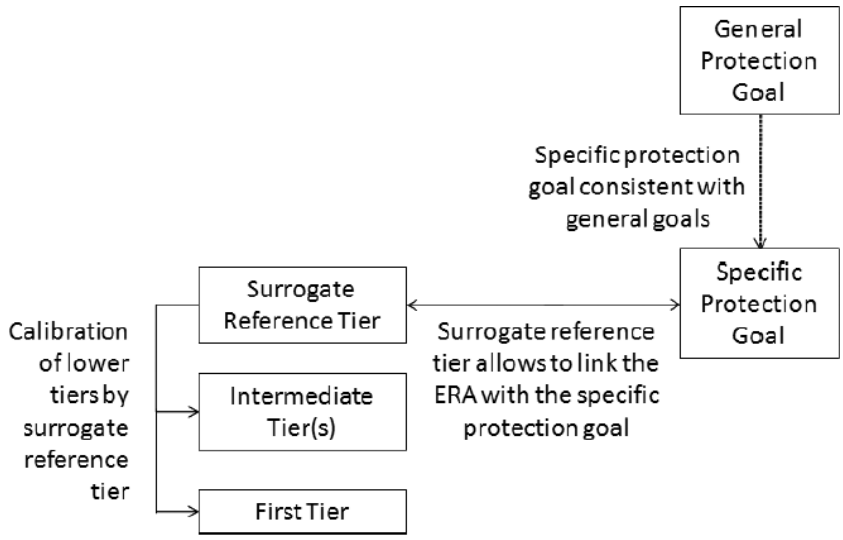

Figure 11: Illustration of the relationship between tiers of the risk assessment process and protection goals, in the approach used by the PPR Panel (EFSA PPR Panel, 2010a)

The risk assessment of PPPs for in-soil organisms should be performed over different spatiotemporal scales (in-field, boundary-scale, off-field). This is in contrast to what is currently done, where only the 'in-field' area is considered. Since an 'action at a distance' is not expected to occur for in-soil organisms on larger scale within relevant timeframes (see Section 6.1), a landscape-scale assessment (multiple field scales) may not be necessary. For many in-soil organisms, recovery will be driven mainly by small-scale migrations and reproduction within the field (see Section 3.2). Nevertheless, for some groups, external recovery from adjacent off-field areas and from field-boundaries may be of relevance for the assessment. The risk to in-soil organisms should therefore be assessed both in-field and off-field and assessment may consider processes at the field-boundary scale (see Section 6.1). In the latter case, this would be done using spatial population modelling to take spatial dynamics at this scale into account. Note that this does not preclude the use of spatial models in-field if local spatial dynamics are thought to be important in the demography of the population. So, the actual reference tier for in-soil organisms (for organisms with either high or low dispersal ability) would be the soil-organism community present at the field scale and influenced by temporal and spatial processes at the field-boundary scale.

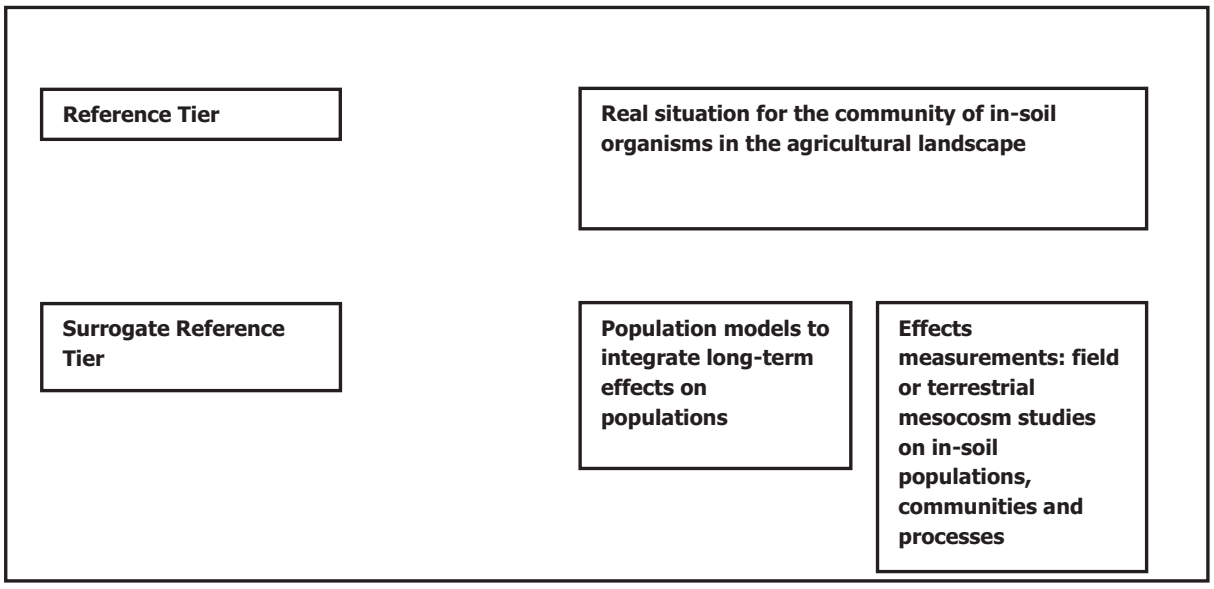

Figure 12: Reference tier vs surrogate reference tier in the risk assessment of in-soil species

In the current risk assessment scheme, the highest available tier is represented by a full fauna field study performed at a local scale. However, in-soil organisms may be exposed to multiple stressors due to sequential as well as simultaneous use of different pesticides and other agricultural practices, which cannot be exhaustively studied in field experiments. Therefore, a combination of assessing the effects both at the local scale – through testing the toxicity of PPPs on in-soil communities – and at a larger scale covering field-boundaries and adjacent off-field areas – through modelling long-term exposure of single species populations integrating all relevant stressors – is proposed as a SRT for in-soil species to assess population-level effects (Figure 12). Even though a larger scale approach (landscape) is not needed for the majority of in-soil organisms with a range of movement that is very restricted compared with field size, for relevant groups the movement between field and boundary may need to be included in the risk assessment.

In this context, it can be assumed that a suitable field study or a mesocosm study (e.g. a terrestrial model ecosystem (TME) with adequate dimensions) can act as a SRT for the assessment of community effects at a field scale. These studies address community composition, population dynamics, indirect effects (predation or competition effects), chronic exposure (eventually repeated exposure), interactions between and within species and exposure mimicking the actual field situation. Please refer to Section 9.7 for recommendations on how to set up of appropriate field test and semifield mesocosms (e.g. TME).

Long-term dynamics at population level over one or more seasons, embracing population growth and spatial dynamics in-field, as well as recolonisation, should be tackled by modelling approaches (combining spatial and temporal population models). The rate parameters of these processes may depend on the field size, the spatial configuration of the crop, the PPP application scenario (in rows or over the entire area) and the existence and dimension of field boundaries and adjacent off-field areas. To assess population-level effects, models for different ecological and agricultural practice scenarios should be developed for relevant key species, with different vulnerability components, and further validated.

In all cases when population modelling is used, the development of suitable baseline scenarios against which to evaluate the effect is critical. However, depending on the SPG it is not always easy to determine which baseline will provide the most sensitive outcome (see Section 4). For this reason, we recommend that in all cases a representative range of baselines should be used from intensive agricultural systems to extensive sustainable systems, and natural conditions in the case off-field or boundary-scale scenarios are needed.

7.3. Surrogate reference tier (SRT) and the systems approach

Current practice in prospective ERA is to conduct the exposure and effect assessment for one PPP at a time. An important question is whether the chemical-by-chemical approach in the current prospective ERA for PPPs is sufficient also to prevent cumulative risks from exposure to different PPPs, as well as to predict ecological recovery. To determine this, the impact of multiple stressors on the state of the population needs to be taken into account when assessing a particular PPP impact. Thus, due to the complexity of ecological systems and the need to evaluate direct and indirect effects and recovery in spatial and temporal dimensions, a systems approach is considered appropriate by EFSA (EFSA Scientific Committee, 2016a). In this context, a *systems approach* is defined to mean taking into account the range of factors considered to potentially interact and affect the result of the risk assessment. For example, this would include multiple applications and non-chemical stressors as they might affect the organisms considered in the assessment. It may also include indirect effect and abiotic factors. The surrogate reference tier (SRT) for this type of assessment would thus be a fully implemented ecological model system including the important factors identified.

In many other systems (e.g. non-target arthropods, aquatic systems), the *systems approach* is needed owing to the impacts of both spatial and temporal drivers of population change. Spatial drivers, in particular 'action at a distance' are relevant for those groups of organisms (EFSA PPR Panel, 2015a; EFSA Scientific Committee, 2016a). In soil, the scales and rates of movements are smaller and thus the primary drivers considered are temporal drivers of population change, i.e. the vital rates. This means that recovery would be primarily driven by internal population growth rather than external migrations (see Section 3.2), and that the measurement endpoint in focus is the long-term population growth rate.

In order to adopt a systems approach and to integrate this into the risk assessment, several steps need to be taken:

1) Relevant taxa and focal cropping systems need to be identified to create relevant scenarios. These species need to cover those where population impacts and recovery can be related to the SPGs;
2) The normal operating range of relevant taxa needs to be identified (bearing in mind that this may vary in time and between different ecosystems). This is used to establish baselines against which the system with the addition of the regulated pesticide can be assessed. These baselines would need to be established for the range of scenarios needed to represent the range of conditions for which the assessment should cover (e.g. low input and high input agro-ecosystems);
3) Good mechanistic effect models, which are both manageable and realistic enough, will need to be developed. To assess effects on other species in an ecological network requires food-web modelling (De Ruiter et al., 2005a,b). However, the use of food-web models for assessment would require that they are predictive, and that their predictive quality has been proven in independent experiments. Hence, although food-web models are conceptually suitable and appropriate, parameterisation and uncertainty of predictions are challenges in their application in risk assessments (see e.g. De Ruiter et al., 1998, 2005a,b; Traas et al., 2004; Baird et al., 2011; De Laender et al., 2011). For community-level assessment, recourse must therefore be made to field studies (see Section 9.7). Note also that the longer time-frame for field-study assessment provides the potential to detect delayed community or life-history effects, e.g. as a result of reproductive impacts. However, in terms of understanding the case-specific results of field studies, food-web models may play an important role. In contrast, population models are relatively easy to develop and require fewer case-specific data. Hence, for assessment of long-term impacts the use of population models is proposed.

For in-soil organisms, it is advised to take the aspects that affect life span into account, rather than large-scale spatial dynamics. For some organisms, vertical movements in the soil profile might be of relevance to assess exposure to PPP. Therefore, for in-soil organisms, there are only a limited number of aspects to consider in terms of their impact and timing. These are:

- The regulated stressor of interest and its intended use;
- Abiotic conditions, e.g. temperature and moisture, as they affect population-growth rate;
- The reproductive profile within a season of the relevant taxa;
- The mortality profile within a season of the relevant taxa;
- Individual growth and development;
- Individual vertical movement within the soil profile, if relevant;
- Individual toxicokinetics and toxicodynamics of the active substance, in combination with varying exposure in the soil profile;
- Impacts of non-regulated stressors, probably primarily impacts of agricultural management;
- Other regulated stressors, i.e. other pesticides, GMO crops and biocides, as relevant.

The models to be developed do not need to take every possible management scenario into account. In edge-of-field surface waters there are typically 2–3 pesticides dominating the mixture in terms of toxic units (see e.g. Liess and Von der Ohe, 2005; Belden et al., 2007; Schäfer et al., 2007; Verro et al., 2009). Consequently, when addressing cumulative stress of pesticides in ERA, it seems cost-effective to focus on those pesticides that dominate the exposure in terms of toxic units in the relevant medium (e.g. > 90%). However, it is important that a range of scenarios altering potential vulnerability of populations is taken into account (e.g. highly stressed populations may be more vulnerable to further stressors).

Information on the distribution of crops in agricultural landscapes and frequently occurring pesticide combinations may be derived from existing databases (e.g. databases under the EU subsidies scheme and databases from EU pesticide usage as collected within the frame of the Sustainable Use Directive, Garthwaite et al., 2015). This information may be important input for population models to evaluate effect periods and recovery times following pesticide stress in a realistic agricultural landscape context (e.g. Focks et al., 2014; Topping et al., 2016).

7.3.1. Population modelling for lower tier assessments

The use of population modelling including all relevant environmental and ecological parameters, is designed to cover two important endpoints in the risk assessment, i.e. long-term population effects

and spatial distribution effects. These endpoints are considered over multiple years and would be expressed as a change in distribution of the population over an area modelled and a change in density of area occupied (Høye et al., 2012; EFSA PPR Panel 2015a). To use the modelling in this way, it will be necessary first to have decided exactly what criteria would be applied to the data from population model(s) in order to assess whether or not the relevant SPGs were achieved (or whether or not they were achieved to a sufficient degree). In this case, application of the models does not suffer from the same issues as field studies or TMEs, i.e. practical issues of measurement endpoints interpreted against minimum detectable differences in field data; outputs from the models can be very precise. However, modelling involves other uncertainties (see Section 7.7.2).

One possibility would be to use the modelling to calibrate the toxicity tests for lower tiers. The outcome of the population model(s) would be dependent on parameter values used for toxicity and other chemical properties in combination with the relevant use and environmental scenarios (the regulatory scenario (EFSA PPR Panel, 2014b)). Provided that the model(s) were easy enough to run, it should in principle be possible to establish the highest toxicity input that would lead to acceptable outcomes for any particular substance. However, by doing this, there is a risk of confounding uncertainties associated with different endpoints and complicating the use of the model at higher tiers. Therefore, we recommend that the modelling and standard toxicity testing are seen as parallel and complementary activities.

7.3.1.1. Practical application of population modelling in lower tiers

Lower tiers should in principle be more conservative than higher tiers, and the tests should be easy to carry out. The use of complex population modelling in lower tiers seems contradictory to these principles, but need not necessarily be so. To use the models developed as one component of the surrogate reference tier in lower tiers, three main criteria need to be met:

1) The models must be standard, agreed models for focal vulnerable species where the behaviour is known and trusted without the option to alter model behaviour.
2) The scenarios used should be standard scenarios.
3) Inputs to the models must be simple, ideally the same data as used in, or coming from, standard lower tier tests (toxicity and use information).
4) For the lowest tier, the use of dynamic population modelling is not suggested, rather a set of look-up tables based on the results of standard population modelling of a range of standard scenarios and toxicities should be created. This look-up table can be used as a lower level screening (see Section 7.3).
5) Refinement of the model in terms of specific exposure scenarios will require running the dynamic model, and is thus described as subsequent tier (see below).

If these criteria are met then the results of the models can easily be interpreted as standard outputs, to which suitable standard assessment factors can be applied. This approach is very much parallel to the idea of FOCUS scenarios in aquatic exposure assessment and would mean that the models could be run by anyone with a short training in model usage, but the outputs could be interpreted easily by anyone.

To further streamline the assessment at lower tiers and negate the running of the model as part of the assessment, a look-up table for model results could be used. This was also suggested for non-target arthropod ERA (EFSA PPR Panel, 2015a). Here, a very wide range of standard scenarios (landscapes, toxicity and intended use) would be pre-run and evaluated. These scenarios should cover the range of possible uses, toxicities and modes of action. The lowest tier assessment would then be made by matching the substance to be assessed to the one of standard inputs and using the pre-run scenario results to determine the risk. The advantage of this method would be that initial screening for long-term population impacts, both spatial and temporal, would be very fast and many products could pass this part of the lower tier assessment without the need to run models. Since standard scenarios would need to be developed as part of the ERA guidance in any case, the additional resources needed to run different toxicity profiles for each scenario to create the look-up table would not be very significant.

7.3.1.2. Refinement of population modelling

If a lower tier population modelling screening is failed, refinement of the modelling requires running the models with altered inputs. There is no expectation that the models themselves will be altered as part of this process because to do so would require further tests and agreement of the altered model

by regulators. This would be a complicated and time-consuming procedure and leads to the need for regulators to be able to assess impacts of the model changes on the ERA outputs. Hence, it is proposed that the only refinements allowed would be of toxicology and exposure, e.g. more accurate toxicity inputs, more realistic use or more realistic exposure.

7.4. Recovery

Recovery can be assessed at the levels of individuals, populations, communities, or functions. In broad terms, recovery can be thought of as the return of an ecological entity (e.g. structure such as abundance, or function such as an ecosystem service) to its normal operating range (sometimes referred to as baseline properties), having been perturbed outside that range by a stressor (or multiple stressors). In order to assess recovery, it is first necessary to define what the normal operating range of the ecological entity and/or process is (EFSA Scientific Committee, 2016a).

Recovery can be classified into two main types, depending upon whether it occurs *in situ* (internal recovery) or via dispersal (external recovery). Both types of recovery may be exhibited by the same ecological entity (e.g. at different stages in a species' life-history). However, those organisms more dependent on external recovery will require larger scale (in both time and space) to represent their systems adequately.

EFSA recommends a systems approach in the cases where recovery is assessed (EFSA Scientific Committee, 2016a). This is due to the need to consider spatial dynamics resulting in action at a distance; hence, evaluating recovery at too small a scale may result in erroneous conclusions (Topping et al., 2014). The systems-level approach takes changes in time and space over a larger scale into account, thus subsuming recovery under the long-term impacts on the overall system state (e.g. represented by population size). If initial effects are considered tolerable, recovery can be considered as an essential and integral dynamic of any system subject to regulated stressors, but may not need to be taken into account explicitly if long-term system state is used for ERA.

According to (EFSA Scientific Committee, 2016a), for any (combination of) experimental or modelling approach to show that there will be actual recovery under realistic conditions of use, this approach needs to consider:

i) the properties of the types of potential stressors (including the timing of applications relative to life-history stage, the number and frequency of applications of the same PPP and the cumulative risks of exposure to multiple PPPs);
ii) direct and indirect effects (species interactions);
iii) the relevant taxa and their traits, e.g. related to demography, dispersal and foraging behaviour as well as adaptation to potential stressors;
iv) the specific features of the landscape, i.e. variations in land use, and the types, spatial distribution and connectivity of habitats.

Due to lack of mobility, most microorganisms and many soil invertebrates will primarily depend on internal recovery, and assessment of external recovery will therefore be unnecessary. However, in a few cases, landscape effects (see Section 6.1) will need to be taken into account, and the use of small plots for experimentation needs to be critically assessed as to whether it meets the criteria listed above.

The tools used to develop the systems approach are mechanistic models for prediction, experimentation, monitoring, and expert elicitation. Experimentation usually involves semifield and field studies, which are primarily used for evaluating community interactions, and experimentation and monitoring are employed as a reality check and to guard against unexpected effects.

There exists a number of potential modelling approaches to assess recovery (please refer to EFSA Scientific Committee, 2016a). However, employing these approaches to develop systems models entails a high demand for data and expert skills for both the development and validation of potential models, especially in cases where external recovery is an important part of the dynamics. Population models that do not need to take spatial dynamics into account will therefore be easier to develop and test, and could be applied more easily to less mobile in-soil organisms, to incorporate the long-term effects and multiple stressors.

7.5. Addressing uncertainty

Two areas where uncertainty needs to be addressed are calibration (Section 7.6) and treatment of additional uncertainties (Section 7.7). Calibration is the problem, when only lower tier effects

measurement data is available, of addressing uncertainty about what the outcome of the effects measurement component (field or mesocosm study) of the surrogate reference tier (SRT) would be. Even when highest tier effects data are available for an assessment, however, there are likely to be additional uncertainties that need to be addressed, for example sampling variability for a field study/ mesocosm or uncertainties affecting the population modelling.

The EFSA Scientific Committee (2015) draft 'Guidance on Uncertainty in EFSA Scientific Assessment' provides specific guidance on the treatment of uncertainty when standardised assessment procedures are being developed. In particular, it is necessary to identify and to describe all the uncertainties that affect assessments for which a standardised procedure is being developed. Methods are provided to assist with this task. The standardised procedure should include allowance for as many sources of uncertainty as is feasible. This reduces the burden for subsequent applications of the procedure as those applications need only consider uncertainties that were not already taken into account.

7.6. Calibration of lower tier effects measurements

In current lower tier risk assessment for in-soil organisms (see Section 2.1.4), a trigger value is used to address uncertainties relating to toxicity assessment. The risk assessment works by calculating the toxicity-exposure ratio (TER) and comparing it to the trigger value. In the context of protecting all in-soil species on the basis of current tier 1 tests, the Panel is not aware that a transparent rationale has been provided for the current trigger values, e.g. five for chronic effects, when included in the Regulation 546/2011. Christl et al. (2015) studied the relation between laboratory and field study outcomes for earthworm reproduction testing and suggested that the trigger value of 5 is sufficient to cover the ratio of lab to field no ecotoxicologically adverse effect levels 1 year after intended uses. Heimbach (1997) reported a similar finding for ratios of EC_{50}'s based on a smaller data set. However, the calibration exercises by Christl et al. (2015) and Heimbach (1997) were both in the context of risk assessment where SPGs had not been defined. Neither exercise addresses the issue of protecting all in-soil species at the desired SPG level.

Computing the TER and comparing it to the trigger value of, e.g. five is equivalent to dividing the TER by an assessment factor (AF) of five and comparing the result to one. This is also equivalent to the approach used in aquatic assessment (EFSA PPR Panel, 2013): the toxicity measurement is divided by the AF to obtain the 'regulatory acceptable concentration' (RAC), which is then compared with the predicted exposure. The rest of this section uses the language of assessment factors but it should be understood that the resulting overall AF to be applied can always be implemented instead as a trigger value.

The core problem for calibration and treatment of additional uncertainties is to decide how big the overall AF should be and how the AF should change as more information becomes available to the assessor.

When surrogate reference-tier (SRT) effects measurement data are available, the overall AF only needs to address the additional uncertainties. When standard tier 1 data are available, the overall AF also needs to address uncertainty about what would be the outcomes of the field or mesocosm study if it was carried out (i.e. extrapolation lab to field), (please see Section 7.8 for a schematic risk assessment flowchart). This is the main calibration problem. If laboratory tests are available for additional species (intermediate tier A) or some test data with assemblages of natural communities are available (intermediate tier B), uncertainty about the first component is expected to be reduced and this may lead to a change to the overall AF. Probabilistic modelling of uncertainty as the basis for assessment factors.

One approach to obtain an AF addressing multiple sources of uncertainty and variability is to determine a suitable AF for each source and then to multiply them to obtain the overall AF. This has the apparent advantage that toxicity and exposure can be separated and addressed by separate AFs and that those AFs may themselves by obtained by multiplying individual AFs addressing particular sources of uncertainty. EFSA PPR Panel (2015a) discusses the problems with this approach: it lacks a sound theoretical basis and tends to lead to an overly conservative result.

Probabilistic modelling of variability and uncertainty is suggested by EFSA Scientific Committee (2015) as the best approach to deriving AFs. WHO/IPCS (2014) provides an example of the approach in the context of human risk assessment for chemicals. For in-soil risk assessment, variability of exposure is already addressed by targeting the 90th percentile (see Section 8). In this approach, interspecies and interchemical variation of sensitivity should be described by a statistical model. The available data and knowledge should then be used to determine a probability distribution that

represents the overall uncertainty about parameters in the statistical model. From the model and distribution for the parameters, a probability distribution could be deduced that would quantify risk, for a substance being assessed. That distribution quantifies how much the TER might change if the uncertainties were resolved. It specifies the probability of each possible value for the TER; how likely that value is. The overall AF would then be the quantile of that probability distribution that provides sufficient certainty that allowance has been made for uncertainties. The decision about what quantile to use is primarily a risk-management issue. The probability distribution representing combined uncertainty is most easily obtained by first determining a probability distribution representing each individual uncertainty and then combining those distributions using the mathematics of probability, taking account of any dependence between uncertainties. The practical tools for determining distributions for individual uncertainties are statistical analysis of data and expert-knowledge elicitation (see EFSA, 2014b) in situations where suitable data are not available. Bayesian graphical/network models (for example, Gelman et al., 2013) provide a framework for expressing the relationships between uncertainties and Monte Carlo is then the natural practical tool for computing distributions for combinations of uncertainties.

Where possible the real-world performance of any assessment factor should be validated. However, it may not be possible to do so. The probabilistic modelling approach incorporates the information provided by data which are used in building the model and combines uncertainties rationally. Consequently, it does not lead to combining the worst case of each individual uncertainty in order to arrive at the assessment factor.

7.6.1. Probabilistic modelling for calibration of lower tier effects measurements against the highest tier (field or mesocosm study)

In order to make this kind of calibration when developing the guidance, it will be necessary to have first decided exactly what criteria would be applied to the data from the field study or mesocosm component of SRT if such were to be available for the substance being assessed. The purpose of those criteria would be to assess whether or not the relevant SPGs were achieved (or whether or not they were achieved to a sufficient degree).

The problem is then to decide how much the outcome (as a concentration) of some battery of lower tier tests might differ from the highest concentration that would lead to acceptable outcomes from the field study or mesocosm component of the SRT. Uncertainty about the ratio between the two concentrations would be represented by a probability distribution and the distribution would depend on which tests were included in the battery. For example, the battery might consist simply of the standard required tier 1 tests or it might also include similar tests for additional species or some more sophisticated testing. The distribution would also depend on what method is used to derive the single concentration representing the outcome of the battery of tests. For example, this might be minimum concentration from all the tests or it might be some other statistic such as the geometric mean if that were found to be a better predictor for the field study or mesocosm outcome.

The approach advocated here and in EFSA PPR Panel (2015a) is to use a statistical model, most likely a Bayesian graphical/network model, to obtain the distribution representing uncertainty about the ratio of lower tier outcome to acceptable concentration in field study or mesocosm. The fundamental basis of this statistical modelling approach is the 'random chemicals' viewpoint of Cooke (2010): the substance being assessed is modelled as although it were randomly selected from a population of relevant chemicals. The interchemical variability of the ratio is a primary source of uncertainty for a new substance because the location of the substance in that distribution is unknown. The statistical model describes how the ratio varies between substances and learns about that variability from relevant data and/or expert judgement. Residual uncertainty about the variability makes an extra contribution to uncertainty about the ratio for the new substance.

In practice, there are several reasons why the statistical model needs to do more than just describe variability of the ratio. First, data from highest tier field studies/mesocosms, to be used for calibration, will not actually provide the highest concentration, for each substance, that leads to acceptable outcomes. Instead the data will provide information about some, possibly limited, aspect of dose–response. The statistical model therefore needs to include a dose–response component so as to be able to make a relationship between the data from a study and the highest acceptable concentration. Secondly, the model must include components for intra- and interspecies variability of dose response. Thirdly, rather than build a different statistical model for each battery of tests, it is preferable to build a single statistical model that is capable of quantifying uncertainty for many batteries of tests.

Consequently, the model needs to include components corresponding to the different tests and species that may be tested. An advantage of including a component for interspecies variability in the model is that there is no need for separate species-sensitivity distribution (SSD) modelling and calculations. The model itself will show how the AF should change as additional species are tested.

The way in which, for example, interspecies variation would enter into the statistical model would be through variation in dose–response parameters between species. Similarly, differences between lab and field would be handled by modelling differences between lab and field dose–response parameters. EFSA PPR Panel (2015a), in particular Section 5.5.6 and Figure 19 therein, provides more detail on statistical modelling of relationships between dose–response parameters for different species and tests.

Each of the components in the model will vary between substances and the model needs to describe this variability. By describing variability of each component, the model also describes variability of the ratio of lower tier outcome to acceptable concentration in field study or mesocosm.

This kind of modelling is a natural extension of the basic SSD model (for example, Aldenberg and Jaworska, 2000), which addresses interspecies variability. EFSA (2006) provided a simple way to incorporate interchemical variability into the SSD model. EFSA PPR Panel (2015a) summarises subsequent developments and recent further developments incorporating dose–response and dynamics into models of interspecies and interchemical variability are provided by King et al. (2015a,b). All these models are specific instances of the wider class of hierarchical random effects models. In that class of models, Bayesian networks and graphical models (for example, Gelman et al., 2013) have particular potential for this kind of application. They provide a formal mechanism, supported by theory, for combining expert judgements with data and quantify uncertainties using probability. This is advantageous for decision-making applications of statistical modelling.

Available data relating lower tier test outcomes to each other and to field study/mesocosm outcomes would be used to 'train' the model, in effect, to learn about the interchemical variation. The data providing information, directly or indirectly, about the interchemical variability of any particular component need to be matched in terms of substances in order to obtain real information about interchemical variability for the component. If enough such data are not available, it will be necessary to use instead information obtained by expert knowledge elicitation. The statistical model is needed whether or not the data are available. It provides a structure for breaking the overall uncertainty down into individual sources of uncertainty and for quantifying the overall uncertainty implied by quantification of individual uncertainties whether those are quantified by statistical analysis of data or by expert judgement.

It is not clear that sufficient data are currently available with which to undertake the full calibration process. Mesocosm data are available for only a few substances. It is anticipated that earthworm field-study data exist for a sufficient number of substances, together with matching tier 1 effects data, to model statistically the relationship between earthworm tier 1 and field studies. It should be possible to fill some data gaps. If sufficient data are not available, it will be necessary to obtain further information by expert-knowledge elicitation. However, it would be preferable to have more data, especially mesocosm data for many more substances. There is also an issue about the design of highest tier field studies or mesocoms to be used for calibration. The calibration requires an understanding of the relationship between tier 1 and highest tier outcomes for the same substance. As discussed above, the issue of dose response is unavoidable. Even if it were reasonable to assume that dose–response family and slope are the same in the lowest and highest tiers, there would still be a need for some source of dose–response knowledge. Therefore, for data to be used for calibration, some substances need to have been measured in both tiers and dose–response knowledge is needed from at least one of the tiers. That might seem to suggest some possibility to do without dose–response information from the highest tier. However, relatively little calibration information would be obtained from a highest tier study carried out at a single concentration unless it happened to produce effects in the region of 50% as otherwise it would need a lot of power to distinguish small effects from 0% and large effects from 100%. Much more information can be gained from a study which has at least two concentrations and a measurable difference in effects at the two concentrations and such a study can also be used directly to model dose response. The possible exception to this reasoning would be if it were reasonable to make the additional assumption that dose–response family and slope are the same for all substances. Then, there might be valuable information from single concentration highest tier studies if they provided a good mix of large and small effects.

The trained statistical model could then be used to make a probabilistic quantification of uncertainty, for any battery of tests and choice of statistic to compute from the test outcomes, about the ratio of lower tier outcome to acceptable concentration in field study or mesocosm for a new

substance. In principle, a suitable statistical model could also be extended to take into account any knowledge about chemical properties, mode of action, and pathways to effects of the substance being assessed, which is judged to be informative about the effects to be expected, for example.

In principle, if sufficient data were available, the statistical model could be used to help decide which species it would be most useful to test. If sensitivity of one species group can be very different from the sensitivity of another, then a large assessment factor will be needed if both are not tested. Given data for many substances on a wide range of species, one could use the statistical model to discover how the size of the AF needed would depend on the choice of species to be tested. In the absence of such a data set, expert judgement would play a large role in quantifying the model. Then a more direct approach to choosing test species is to decide *a priori* which group of species when tested are most likely to cover well the sensitivities of all untested species of interest without needing to a apply a very large assessment factor.

Most of the reasoning in this section applies to any alternative approach to calibration. If a statistical modelling approach was not used, the group of experts deciding the assessment factor or trigger value would still need to reason about the same issues. They would not be able to so without implicitly or explicitly considering dose response, interspecies variability and variability between substances.

7.7. Additional uncertainties

7.7.1. Addressing uncertainties affecting the effects measurement component of the surrogate reference tier (SRT)

As well as addressing the calibration problems as described in Section 7.6, an AF can also be used to address additional uncertainties that relate to the SRT and that are anticipated at the time when the assessment factor is being determined. For example, these might include known weaknesses of field study/mesocosom protocols. In principle, these might be addressed when determining the criteria for evaluating the acceptability of field study/mesocosm outcomes. For example, if those outcomes were subject to significant statistical uncertainty, one might choose to use an upper confidence limit for effects if such a limit was available. However, this would be analogous to choosing an AF for this particular source of uncertainty and it might be preferable to address the problem by adding a suitable component to the statistical model used for calibration described in Section 7.6.1. For the example given, one would include an interstudy component in the statistical model.

7.7.2. Addressing uncertainties affecting population modelling

Uncertainties relating to the population model(s) themselves and that are not substance-specific should be addressed by the use of standard assessment factors. These assessment factors should cover uncertainties related to both model inputs and model outputs.

For inputs, the primary uncertainty is the relationship between the toxicity measured by the standard tests and the sensitivity of the species for which the model is considered to represent. This means that part of the assessment factors (those related to species sensitivity) used in lower tier toxicity tests should be applied to the toxicity input. In this case, the assessment factor should be applied by increasing assumed toxicity (or application rate).

For outputs, there are other model uncertainties which are related to the way in which the model is constructed and the mechanisms represented and their interactions. Uncertainly analysis should be available because the model should be constructed following good modelling practice (EFSA PPR Panel 2014b), but since it is unclear how uncertainties will propagate through the model those uncertainties not linked directly to inputs should be dealt with using assessment factors on the outputs. Here, extended sensitivity analysis also covering scenario inputs can help to link uncertainty in complex inputs and model structure to the scale of effects seen in model outputs. For example, Dalkvist et al.. (2009) showed that population-level impacts in a modelled vole system were scaled non-linearly to toxicity, and other ecological factors had equal or greater impacts. Similarly, impacts of changing farming or landscape assumptions were of paramount importance in assessing the impact of an endocrine disruptor on hare populations (Topping et al., 2016). For output uncertainties, the AF should be applied as a reduction in the level of effect that is acceptable.

7.7.3. Uncertainties specific to a particular assessment

In principle, uncertainties common to all assessments should have been addressed by Sections 7.6.1, 7.7 and 7.7.1. There may exist additional uncertainties for any particular assessment, and provision should be made in the guidance for addressing them. Consideration should also be given to providing, in the guidance, a checklist of recognised, possible additional uncertainties. The checklist would make it easier for assessors to evaluate the need to address additional uncertainties of this kind.

7.8. The risk assessment flowchart

The risk posed by PPPs and their active substances to in-soil organisms – soil fauna and microorganisms – is currently assessed separately for each 'group' of the in-soil organisms' community (European Commission, 2002). Macrofauna (earthworms), mesofauna (collembola and mites) and microorganisms are considered independently in their own risk assessment schemes from lower to higher tiers, depending on which group was indicated to be at risk at the first assessment step. Since even at the highest tier in field trials (surrogate reference tier, see Section 7.3) it is currently seldom attempted to assess the risk for the soil community as a whole – rather, only earthworms or collembola are investigated – the interactions between the components of the soil biocoenosis are not addressed so far, and the detection of possible indirect effects of use of PPPs is not possible.

The proposed risk assessment flowchart (see Figure 13) accounts for the fact that the evaluation of indirect effects is part of the data requirements (EU 283/2013 and 284/2013, see also Section 2). Accordingly, it is advised that higher assessment tiers (surrogate reference tier) should address interactions between species and indirect effects via food-web disruption – besides effects on species/groups that are not tested at lower assessment tiers but are believed to be at risk (see Section 9).

The proposed risk assessment scheme, shown in the flowchart, has two components: (i) measurements of effects in laboratory, field or semifield studies; and (ii) assessment of long-term effects using population modelling. This second component is included to ensure that the lower tier assessment is the most conservative, which may not be the case if long-term effects are not included. The principle of the scheme is that the active substance or PPP must meet acceptability criteria with respect to both components.

As first step, as indicated in the scheme, the effects of active substances or PPPs on in-soil organisms are investigated in simple laboratory tests. According to the current data requirements (EU 283/2013 and EU 284/2013), tests are currently performed with an earthworm species, a collembola, a mite species and a test addressing the nitrogen transformation capacity of in soils by microorganisms (see Section 2 for further information), assuming that these are appropriate surrogate species and processes. Additionally, to the assessment of active substances or PPP effects on these organisms, the Panel advises to investigate effects on mycorrhiza fungi and on litter feeding organism (e.g. isopods). Please refer to the sections on specific protection goal options (Section 6) and effect assessment (Section 9) for the rationale behind these proposals and for the choice of the appropriate tests, respectively.

If the comparisons of the detected effects in the lowest assessment step with the predicted environmental concentration meet the respective acceptability criteria (trigger value), no further assessment is needed for the first component of the scheme. For the derivation of pertinent assessment factors that address the uncertainties existing when extrapolating from effects detected in the lab to effects on the community of in-soil organisms in the reference tier, please refer to Section 7.6.

If the relevant trigger values are not met at the lowest tier, the risk may be refined by (i) refinement of exposure (see Section 8) or/and (ii) further ecotoxicological testing (Section 7.8.1), which might improve the description of the risk for soil organisms and address specific uncertainties present at the lowest assessment steps.

The second component of the scheme addresses the effect of year on year application of PPPs in a so-called 'system approach' with appropriate population models based on vulnerable focal species (see Section 7.9). A parallel assessment of long-term effects on soil organisms species is needed, since it addresses uncertainties in the risk assessment that have to be addressed already at lower tiers. This is split into two tiers. The lowest tier uses standard inputs to a screening step which is based on conservative population modelling scenarios, pre-run and tabulated as look-up tables using the toxicity data from standard tests applied to models of vulnerable focal species. If this screening triggers concern, refinement of the inputs to the model and running the dynamic model forms a higher tier.

Here, inputs may be refined exposure (e.g. based on specific chemical properties or climatic scenarios) or specific application schedules not covered by the standard scenarios.

If the trigger value(s) for model output are not met at the lowest tier, it may be possible to refine the exposure assumptions used in modelling. The results of modelling approaches assessing the effects of year on year application of PPP on in-soil organisms in a so-called system approach can only be refined to a very limited extent with further ecotoxicological testing at higher tier (e.g. toxicity data for other species). Accordingly, information from higher tier testing with soil-organism communities cannot be used to refine the risk indicated by population models, since these approaches address different uncertainties in the risk assessment, therefore the criss-cross model does not apply to linking population modelling and other effects assessments.

7.8.1. Refined measurement of effects

The highest tier of effects measurement in the flowchart (Figure 13) is a natural assemblage test with soil organism communities in the form a of a TME or field study. As possible intermediate tiers, two types of test set ups are highlighted that may be performed to address specific uncertainties of the assessment before performing a field or semifield tests with intact soil communities exposed to active substances or PPPs.

One type of experiment can be performed to address the possible differences in sensitivity of species belonging to one group (intermediate tier A). Testing singularly more species of microarthropods might reduce the uncertainties regarding the sensitivity of *Folsomia candida* as a surrogate species towards the tested substances. Please refer to Section 9.5 for discussion of the pros and cons of species sensitivity distributions (SSD). Regarding microorganisms, recent developments of functional tests (BIOLOG®, MicroResp®, see Section 9.3.2) give more detailed information on the capacity of microorganisms to degrade different types of compounds, and the effect of pollutants on degradation.

Another type of experiment studies effects on natural communities assembled in a generic microcosm set-up, using segments of the natural community and not just an assemblage of 2–3 species (intermediate tier B). Here, interactions between species and groups of in-soil organisms and the structural and functional responses of endpoints for microorganisms' communities may be jointly investigated. Please refer to Section 9 for the characterisation of these types of tests. However, since experience with this type of assembled terrestrial communities is so far almost completely lacking – and therefore the proper calibration of the outcome of these experiments with the reference tier could be very difficult – this step of the proposed risk assessment scheme is considered to be conceptually relevant but problematic in the implementation until further experience is available. This is indicated by the light shading of this refinement step in the box displaying the 'intermediate tier with assembled communities'.

If the relevant trigger values indicate high risk for in-soil organisms at these intermediate assessment steps (e.g. with SSD or with SSD and microcosms), exposure-refinement options and possible risk management measures might be considered as in the lowest-tier assessment step. If a low risk is indicated, no further refinement steps are needed.

In the case that more information is needed to conclude on low risk for in-soil organisms exposed to intended PPP uses, higher tier assessment steps might be performed. Please refer to Section 9.7.1 for the description of higher tier effect-assessment test options. Given the high natural variability of measurement endpoints regarding in-soil organisms in the field, it is recommended to select test set ups with pertinent statistical power.

Field or semifield tests are performed with the aim of characterising the effects of the active substance in PPPs on the communities of in-soil organisms under realistic conditions. It is recommended to assess jointly populations of macro-, meso- and microfauna and structural/functional responses of microbial communities, in order to be able to detect indirect effects of intended uses of PPPs on in-soil organisms, i.e. disruption of food webs or significant shifts in niche occupancy.

7.8.2. Application of the flowchart

If on the basis of such a surrogate reference tier assessment, it is indicated that the risk is high, exposure refinements or realistic management options might be considered to reduce the risk for in-soil organisms. If such options cannot be implemented, it cannot be concluded risks are low for in-soil organisms under the intended use of the assessed PPP.

Although outside the remit of this Opinion, it is acknowledged that appropriate risk mitigation measures and inclusion of management options might lead to the indication of acceptable risk under realistic conditions. For further information on risk-mitigation measures, please refer to the outcome of the workshop on mitigation measures in agricultural land (MAgPIE – Mitigating the risks of plant protection products in the environment).

This flowchart is valid for the assessment of in-field and off-field areas in agricultural landscapes. Until the implementation of an integrated 'system approach' in the assessment of the risk from PPP use for non-target organisms (see EFSA Scientific Committee, 2016a), it is recommended to assess communities of in-soil organisms with limited mobility present in-field and off-field, – particularly since different SPG options have been proposed for target and non-target areas. Different levels of acceptable effects in-field and off-field might result as trade-offs from agricultural use, making unreliable an *a priori* decision whether the in-field assessment does also cover the off-field environment.

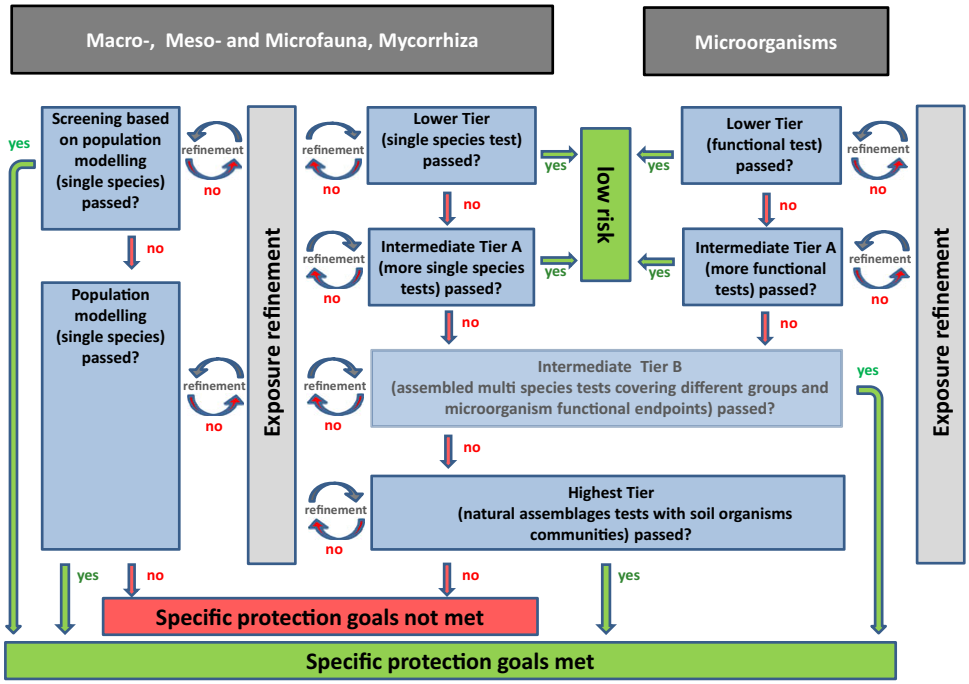

Figure 13: Illustrative risk assessment flowchart for in-soil organisms exposed to (active substances in) plant-protection products. A high risk from an intended use of a PPP is possible unless both the effects measurement and population modelling components indicate low risk. In the lowest tier of effects measurement, organisms like macro-, meso- and microfauna are addressed by single species tests and functional responses are assessed for microorganisms. At the highest tier, effects on different groups of in-soil organisms are assessed jointly, in order to detect possible indirect effects of PPP intended uses

7.9. Identification of vulnerable species/focal trait groups

The information on the traits of in-soil key drivers determining their vulnerability to intended PPP uses is summarised in the following section. The vulnerability of a species to specific plant protection products and other potentially toxic chemicals depends on four major aspects as described by De Lange et al. (2010): probability of being exposed to the toxicant, intrinsic sensitivity to it, possibility of suffering from indirect effects, and ability to recover after a direct or indirect effect. These aspects are closely linked to a number of species traits that could be relevant for the identification of vulnerable species or focal trait groups.

In a field situation, the first step of vulnerability is the exposure of an individual to a toxicant. In general, in the soil environment three routes of exposure are most important: (i) via food, (ii) via direct contact with contaminated soil and/or litter, and (iii) through direct contact with soil solution. Which one of these three routes is most important for a particular species depends primarily on the following three groups of traits:

(1) The prevailing living environment – surface-living vs. soil dwelling vs. inhabiting water-filled soil cavities and pores.
Generally, surface-living organisms can be considered more vulnerable to direct pesticide sprays as they are exposed at the very moment of pesticide application and before any degradation or leaching takes place. Because of this, the exposure rate can be similar as for target pest species but the exposure time via this route might be relatively short. In contrast, soil-dwelling organisms, such as earthworms, microarthropods, millipedes, centipedes or woodlice, are thought to be protected against direct spray by litter and by the top soil layer. However, the exposure time can be much longer than in the case of surface-dwelling animals, depending on the properties of the compound in soil (e.g. persistence, leaching potential) and on the life-form of the organisms. Moreover, the species' behaviour in the profile can lead to vertical movements between soil layers (see Section 7.10.3). For many of these species, both the direct contact with litter, soil or soil solution and food are routes of exposure. The balance between them depends on body cover and size of an organism (see below), with small earthworms belonging probably to the most exposed in-soil organisms due to huge quantities of soil passing although their digestive tract and, at the same time, the direct contact with soil and soil solution (see below). In turn, many smallest soil animals (e.g. nematodes and potworms), most protozoans and bacteria, inhabit small soil cavities filled with water. Many of them can be actually considered aquatic organisms as they cannot live for a prolonged time outside the water-filled soil pores. As they are small, they also have a large surface-area-to-volume ratio (see below) and, hence, the main exposure route is similar to regular aquatic species. For these groups, the main exposure route is probably via the soil solution and transfer of toxicants through the body surface (see Section 7.10.2).

(2) Body cover – hard-bodied vs. soft-bodied organisms.
From this point of view, in-soil organisms can be roughly divided into three groups: hard-bodied invertebrates, such as some mite and collembolan species, woodlice, centipedes or millipedes, protected with a chitin exoskeleton or scales and hairs; soft-bodied invertebrates, e.g. earthworms, potworms and nematodes and microarthropods; and microorganisms, which due to their mostly single-celled structure do not have body cover in the strict meaning of the term (see Section 7.10.2). Body cover determines to a large extent the contact exposure through soil or pore water. For earthworms, which pass large amounts of soil through their digestive tract, plus in anecic species that feed on the leaf litter in the soil surface, the exposure through food might be at least equally important (see Section 7.10.2). Microorganisms are exposed almost exclusively through body surface as there is not much to protect them against absorbing chemicals from the environment and for many this is also the main way of feeding (although many protozoans are phagotrophic, that is ingest small food particles, such as single-celled or filamentous algae, bacteria and microfungi).

(3) Size – the smaller the organisms the larger the surface-area-to-volume ratio.
Surface-area-to-volume ratio is especially important for soft-bodied organisms exposed mostly through the contact of body surface with pore water. In such organisms, the larger the surface-area-to-volume ratio, the higher the bioconcentration rate is. Bioconcentration defines the accumulation rate of a chemical by an organism from its environment. High bioconcentration rate means that in a short time a chemical can reach high concentration in the body of an organism. In case of certain chemicals, in particular organic pesticides, this can lead to body concentrations a few orders of magnitude higher than in the environment. In some organisms, this can directly result in fast acute toxicity; whether this happens depends, however, on organism's physiology and biochemistry (see below) – for example, some microorganisms are able to utilise organic pesticides as an energy source.

Once a species is eventually exposed to a toxicant, its vulnerability is further determined by the next set of traits, linked either to its intrinsic sensitivity to the toxicant or to its recovery potential:

1) Inherent species sensitivity to a particular toxicant or a class of toxicants.
The inherent toxicological sensitivity of a species to a plant protection product is the ultimate and most important trait defining its vulnerability. This trait is intimately related to the toxic mode of action of the compound and to the mechanisms dictating the toxicokinetics (especially its metabolism and excretion components) and the toxicodynamics

processes at the individual, i.e. chemical-receptor interactions and the propagation of effects through molecular networks and over different levels of biological organisation (e.g. cell, tissue, organ and individual) (EFSA Scientific Committee, 2016c).

2) Life history – e.g. semelparity vs iteroparity; short vs long life span; low vs. high reproductive rate, etc.
   The ultimate effect of a toxicant on population depends heavily on inherent population parameters defining its dynamics in the environment. In general, species with high reproductive rates, such as some insects that are able to lay few hundred eggs per season, are considered less vulnerable to environmental perturbations due to their ability to rebuild large populations even within a year (= fast recovery). Most such species are short-lived and semelparous. As such, they die within a season anyway and whether a pesticide treatment has any effect on the population depends more on the timing of the treatment (before or after the reproduction) than on adult mortality. Under normal circumstances, larval mortality is also very high, hence even substantial pesticide-driven mortality in this stage does not necessarily bring a proportional decrease in population size. Such populations are considered to have relatively high 'buffer capacity' for increased mortality as their survival rate is strongly density-dependent. On the other hand, species like some centipedes or earthworms, which are typical iteroparous organisms, can live for a few years, with relatively low annual reproduction. Their intrinsic population growth rate is low and application of pesticides may have much longer lasting consequences. Moreover, due to their long life span, such species may be exposed to toxicants many times in their lifetime and either toxicants themselves or toxicant-driven damage may accumulate in the body.
3) Dispersal potential – i.e. ability to disperse from field-boundary areas to in-field and within in-field
   Dispersal potential of a species or a trait group, together with the spatial structure of field and off-field areas (see Sections 3.2.2 and 6.1), determine to a large extent the external recovery potential of its population or community at a certain crop site. Although soil invertebrates have a lower dispersal ability than other invertebrate groups (e.g. NTAs), movements from seminatural areas like hedgerows and field boundaries towards the in-field treated area have been shown for spiders and even for organisms with low dispersal ability as collembolans, showing that external recovery also plays a role in population/community recovery in the long term. The ability to disperse is also relevant in internal recovery since species/trait groups that were able to keep resistant populations in suitable refugia less affected by the pesticide at the field area (e.g. between-rows in permanent crops or annual crops when pesticide granules are used). This holds true if the animals do not habitually move from contaminated to uncontaminated soil areas (or layers).

To sum up, specific combinations of traits can make a species more or less vulnerable to a plant protection product. In the first step, the inherent sensitivity of different taxonomic groups to a particular pesticide should be assessed. This can be done taking into account the mode of action and, if available, toxicodynamic data of the pesticide. Once this is done, the sensitive groups (species) should be evaluated for potential vulnerability considering the traits described above. Generally, specific combination of traits can be suggested that make a species or group of species particularly vulnerable to a pesticide it is sensitive to: e.g. soft-bodied, small and living in topsoil, has relatively long life span and low intrinsic population growth rate; its dispersal potential is small and it tends to stay in arable areas thought a year. Additionally, predatory species may be particularly vulnerable due to the biomagnification phenomenon.

7.10. Linking Exposure and effects

7.10.1. The criss-cross model

The EFSA PPR Panel, (2010a) described the framework for the risk assessment for aquatic and terrestrial organisms. The risk assessment requires two parallel, tiered flowcharts, one for the effect assessment and one for the exposure in the field. Considering in more detail the interactions between the flowcharts for field exposure and effects, there are only arrows from field-exposure to effect tiers (Figure 14). EFSA PPR Panel, (2012) mentioned that all options for delivery of field-exposure assessments to effect tiers are possible (called the 'criss-cross' model).

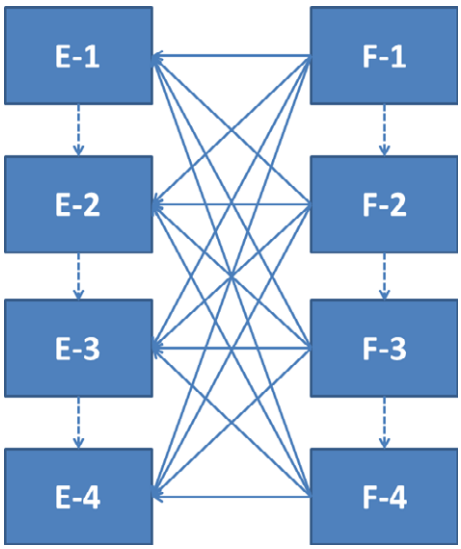

Figure 14: Flowcharts for possible routes through the combined effect and field-exposure. The boxes from E-1 to E-4 are four effect tiers and the boxes from F-1 to F-4 are four tiers for assessment of exposure in the field ('F' from 'field'). Dashed arrows indicate movement to a higher tier. Arrows from right to left indicate delivery of field-exposure estimates for comparison with effect concentrations in the effect flow chart (EFSA, 2013)

The criss-cross model may work well for species with a limited mobility at the landscape level and in the soil profile. Some species, such as carabid beetles, however, live on the soil surface at the adult stage but become soil dwelling as larvae. For adult life stages of those species, landscape-level approaches are proposed for higher tiers of the risk assessment in the Opinion of the PPR Panel on Non-target Arthropods (EFSA PPR Panel, 2015a). Such a higher tier assessment would involve simulating the intended use (i.e. good agricultural practice (GAP)) at the relevant spatial scale for in-soil organisms and including fate and exposure, as well as the ecotoxicological and ecological data as directly simulated components (Topping and Odderskaer, 2004; Topping et al., 2015a). Lower tier exposure assessments are typically based on a single realistic worst-case scenario (EFSA, 2010; EFSA PPR Panel, 2010b, 2012). It is obvious that such lower tier exposure scenarios cannot be used in combination with landscape-level approaches, since such scenarios are not spatially explicit (EFSA PPR Panel, 2014b). If modelling approaches were to include toxicokinetic/toxicodynamic (TK/TD) modelling to take into account vertical movement of PPPs in soil as suggested in Section 9.9.3, then this criss-cross concept would similarly be invalidated. The applicability of the criss-cross model and alternatives will be further elaborated during the development of the in-soil guidance.

Figure 15 shows in detail how the interaction between exposure and effect assessment theoretically works for an arbitrary combination of an effect and a field-exposure tier (by zooming in on an arbitrary combination of an effect and field-exposure tier from Figure 14). The standard procedure in soil ecotoxicological experiments is to use a range of concentrations to derive a concentration–response relationship. Toxicity endpoints within effect tiers have to be expressed in terms of the same type ERC as the endpoints of the field-exposure tiers. This implies that there are two equally important types of exposure assessments required for the risk assessment procedure. The first assessment (in the field-exposure box in Figure 15) involves estimating the exposure (in terms of a certain type of ERC) that will occur *in the field* resulting from the use of the PPP in agriculture. This is part of the field-exposure flowchart (see EFSA PPR Panel, 2012 and Section 8 for details) and is referred to as the predicted environmental concentration. The second exposure assessment (in the effect box in Figure 15) is a characterisation of the exposure (defined in terms of the same type of ERC) to which the organisms were exposed in the ecotoxicological experiments. This second exposure assessment is part of all tiers in the effect flowchart and is the metric that is used to express the effect (the 'C' in the NOEC or EC_x estimate).

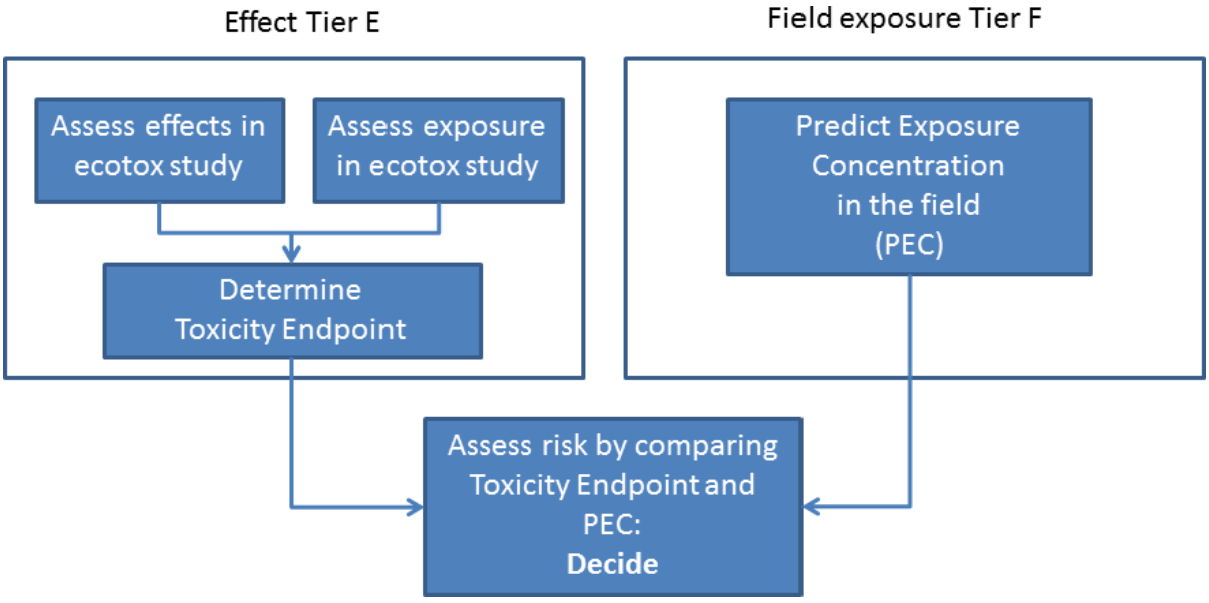

Figure 15: Schematic representation of the two types of exposure assessments that are needed in any combination of tiers of the effect and field-exposure flowcharts

7.10.2. Exposure routes of key drivers

In-soil organisms are exposed to PPPs via a variety of pathways. This is modulated mainly by their morphology (e.g. their body form or the structure of the epidermis), physiology (e.g. the way they take up water, food and oxygen) and behaviour (where they live and move in soil) (Peijnenburg et al., 2012). Moreover, these pathways may vary during the life cycles of some species. The relative relevance of these uptake routes for the body burdens is also dependent on the properties of the chemical (e.g. hydrophobicity) and environmental conditions like soil properties and climate.

The major uptake routes considered for soil organism are:

- Contact with soil, soil pore water and litter (so diffusion into the body via the 'skin');
- Ingestion of food (soil organic matter, litter, bacteria, fungi, prey), of soil particles and soil water;

A usual distinction is made between the so called 'soft-bodied' and the 'hard-bodied' organisms. The former include earthworms, enchytraeids, nematodes, some collembola and insect larvae, whereas the latter are composed by collembolans living in the upper soil profile, mites, insects and the epigeic detritivores – isopods and millipedes – and predators like spiders and centipedes.

For 'soft-bodied' organisms, where water and oxygen is taken up mainly via the skin, soil pore-water is considered the most important uptake route for chemicals (Belfroid et al., 1994; EFSA, 2009c; De Silva et al., 2010; Šmídová and Hofman, 2014; Diez-Ortiz et al., 2015). Ingestion of contaminated food and soil particles and the subsequent absorption of chemicals via the gastrointestinal tract, however, can play a significant role as well (Šmídová and Hofman, 2014; Katagi and Ose, 2015). According to Belfroid et al. (1995), laboratory studies performed with earthworms and a range of PPPs showed that the uptake deviates by a factor lower than two when compared by model predictions based on equilibrium partitioning theory (EPT). However, differences between species with different life-forms should be taken into account. Belfroid et al. (1994) found higher accumulation factors for *Eisenia andrei* (an epigeic species feeding mainly on humic material) than for *Lumbricus terrestris* (an anecic species feeding mainly on plant litter), which could indicate that uptake via soil could be of less importance for anecic species. On the other hand, anecics do ingest large amounts of soil when burrowing and looking for food, and evidence of temporary increase in internal concentration of hexachlorobenzene (HCB) was found in *Lumbricus terrestris* when placed in a new contaminated soil and after the creation of new burrows (Beyer, 1996). Jager et al. (2003) and Katagi and Ose (2015) present two-compartment models for earthworms, including the adsorption of contaminants from the food via the gut. Jager et al. (2003) points to the fact that the contribution of the gut route increased

with increasing hydrophobicity of the chemical. For the tested substance with highest K_{ow} values, the gut route clearly dominated. Moreover, measured concentrations in the worms exceed equilibrium with the soil and bioconcentration increased more with higher Kow than did soil sorption. Interestingly, the rate constant for exchange across the skin in the soil environment is much higher for hydrophobic compounds than an exchange in a 'water-only' situation would predict.

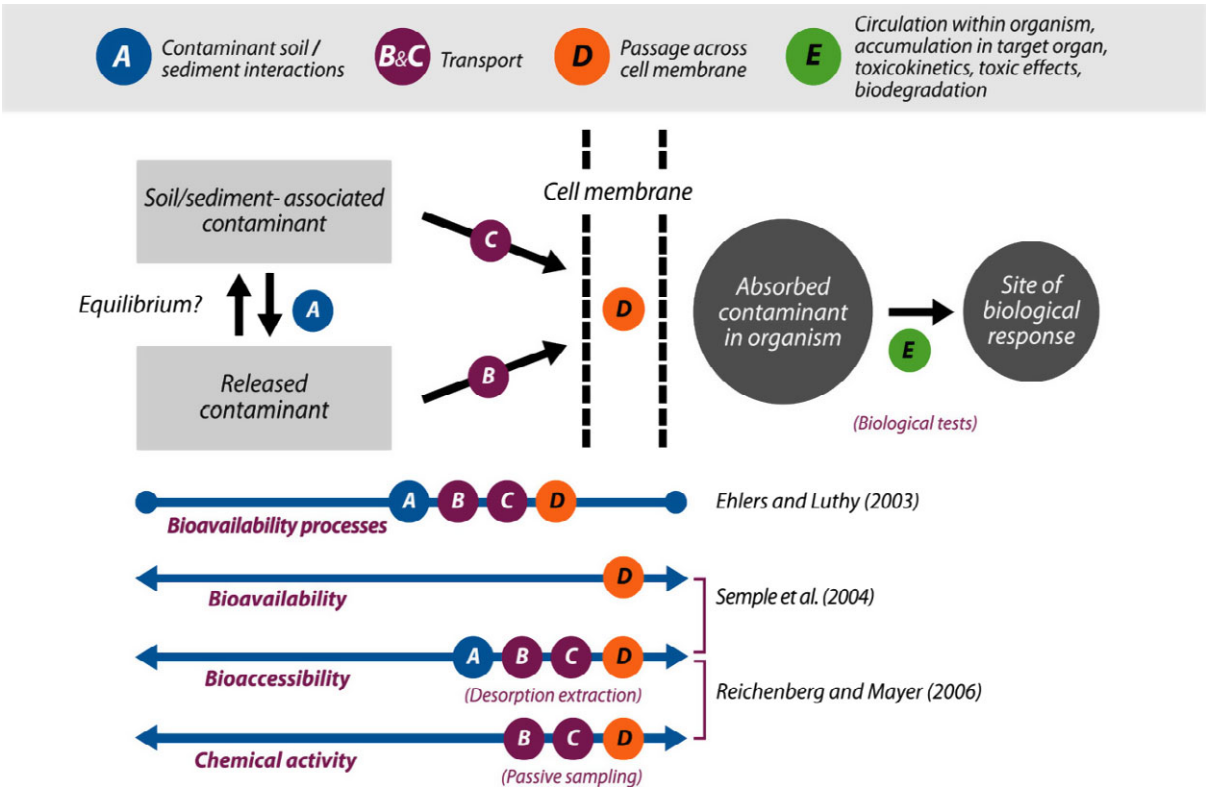

Figure 16: Scientific concepts on the bioavailability of organic chemicals. (Reprinted with permission from Ortega-Calvo J, Harmsen J, Parsons J, Semple K, Versonnen B, Aitken M, Ajao C, Eadsforth C, Galay-Burgos M, Naidu R, Oliver R, Peijnenburg W, Roembke J and Streck G, 2015. From bioavailability science to regulation of organicbchemicals. Environmental Science and Technology, 49, 10255–10264. Copyright (2015) American Chemical Society). Bioavailability can be examined through chemical activity, the potential of the contaminant for direct transport and interaction with the cell membrane (processes B, C and D), or bioaccessibility measurements, which incorporate the time-dependent phase exchange of the contaminant between the soil/sediment and the water phase (process A). Depending on biological complexity, the passage of the contaminant molecule across the cell membrane (process D) may represent multiple stages within a given organism before the site of biological response is reached (process E)

'Hard-bodied' organisms take up water and oxygen via special organs. For these organisms, ingestion of food is also a relevant exposure pathway. However, as expected, assimilation rates of the different food items play a key role in modulating the actual uptake in the digestive tract, and unfortunately not many studies exist on this topic, especially when dealing with microbivores (mainly collembolan and mites that are fungal and bacterial feeders) and predators (especially predatory mites). 'Hard-bodied' organisms can also take up chemicals via contact with soil and/or soil pore water, as demonstrated for collembolans (Gyldenkaerne and Jorgensen, 2000; Fountain and Hopkin, 2005; EFSA, 2009c) and isopods (e.g. Sousa et al., 2000; Santos et al., 2003). The former authors found that uptake from soil pore water was the major route for pyrethroids and dimethoate. Regarding isopods, Sousa et al. (2000) reported that internal concentrations of lindane in *Porcellionides pruinosus* exposed via soil were 25 times higher than when exposed through food, which were due to different degradation kinetics of the active substance in the two matrices. Santos et al. (2003) found that internal body burdens in the same species and also for lindane were better correlated with

concentration in soil extracts than in bulk soil. Similar findings were also reported by Belfroid and Van gestel (1999) for soft-bodied organisms like slugs, where accumulation of DDT via soil was 10-fold higher than via food. Nevertheless, uptake via feeding on the litter layer should not be excluded as an important exposure pathway, especially for very hydrophobic compounds (Van Brummelen et al., 1996). Furthermore, exposure in the litter layer does not occur only via feeding but also via contact with the litter and its water-film. The results mentioned above are based on laboratory studies and generalisations to a real field scenario should be made carefully, as should generalisations to other species, even within the same group of organisms, due to the low number of species tested.

Taking a pragmatic approach in terms of conducting a risk assessment, a major uptake route for 'soft-bodied' organisms (e.g. earthworms, enchytraeids, soft bodied collembola, nematodes and some insect larvae) seems to be the uptake from soil pore water. Moreover, the concentration in pore water is also considered to be the driving factor for uptake and toxicity of pesticides for microorganisms. However, the relative importance of other uptake routes depends on the properties of the assessed active substance and will increase with increasing hydrophobicity of the chemical.

Regarding 'hard-bodied' organisms (e.g. some collembolans, mites, isopods), although contact with soil plays a role, evidences of the importance of soil pore water as an important exposure route to these organisms does also exist. This stresses the importance of considering exposure of in-soil organisms to pore water as well, particularly for compounds that have high water solubility. The Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface waters (EFSA PPR Panel, 2015b) states that the freely dissolved fraction in pore-water of sediment-associated PPPs most likely is the main exposure route for these organisms, although dietary exposure might also play a role.

Regarding uptake via food, namely for those organisms feeding on fungi, bacteria, soil organic matter, and for predatory organisms, not many data are available and the derivation of robust concentrations in these matrices is, for the moment, a difficult task. Furthermore, evidence exists that this uptake route has high relative relevance for compounds with a high K_{ow}.

Particular attention should be given to the litter layer (EFSA PPR Panel, 2010c). Even if the definition of a 'proper' litter layer includes certain stability over time, dead organic matter as plant debris is located on the soil surface of most crop systems during the vegetation period and after harvest. Plant debris constitutes an important food source for anecic earthworms, like *L. terrestris*, and litter dwelling organisms also in non-permanent crops, with consequent relevance as an uptake pathway for active substances, and there is also potential uptake via contact with the litter material. This would be more common in annual crops with low or no tillage and permanent crops. Exposure from the litter should therefore, be taken into consideration, whether from a litter layer or from plant debris on the soil surface.

Table 21: Exposure routes of key drivers belonging to the in-soil organisms

Group affected		**Exposure route**	
		Via contact	**Via food**
Litter dweller			
Feeding type	***Key drivers***		
Litter fragmenters, detritivores	**Macroarthropods (e.g. isopods, millipedes) gastropods (snails and slugs) non-arthropod invertebrates (e.g. earthworms, enchytraeids)**	**Litter layer/water film**	**(fragmented) litter**
Predators	Macroarthropods (e.g. centipedes) microarthropods (e.g. mites)	Litter layer/water film	Prey
(micro) detritivores, grazers, browser	Microarthropods (e.g. collembola, mites), nematodes	Litter layer/water film	Fragmented litter, fungi & bacteria
Decomposers	Microorganisms (fungi, bacteria)	Litter layer/water film	(fragmented) litter
Soil dweller			
Feeding type	*Key drivers*		
(micro) detritivores, grazers, browser	Microarthropods(e.g. collembola, mites), nematodes	Soil and soil pore water	Dead organic matter, fungi & bacteria

Group affected		Exposure route	
		Via contact	Via food
Litter dweller			
Feeding type	***Key drivers***		
Litter fragmenters, detritivores	**Macroarthropods (e.g. isopods, millipedes) gastropods (snails and slugs) non-arthropod invertebrates (e.g. earthworms, enchytraeids)**	**Litter layer/water film**	**(fragmented) litter**
Detritivores	Non-arthropod invertebrates (e.g. earthworms, enchytraeids)	Soil and soil pore water	Soil, dead organic matter, fungi & bacteria
Decomposers	Microorganisms (fungi, bacteria)	Soil and soil pore water	Dead organic matter
Predators	Macroarthropods (e.g. beetle larvae), microarthropods (e.g. mites), nematodes	Soil and soil pore water	Prey
Herbivores	Invertebrates (e.g. snails, nematodes)	Soil and soil pore water	Living plants

7.10.3. Temporal and spatial field exposure profiles for in-soil organisms

In-soil organisms are exposed to a range of different concentrations in the medium where they dwell, when active substances reach the soil after intended uses of PPP. Exposure gradients for in-soil organisms towards the active substance applied are present since the very first entry time of the active substance (a.s.) on or in the soil and these gradients evolve through time. One gradient is spatial, related to the vertical distribution of the active substance in the soil profile; the other gradient is temporal, related to the fraction of the active substance that is (bio)available over time.

Since soil animals move (some more, some less) through the soil profile in a circadian/seasonal rhythm that is modulated by light, temperature, humidity and the food and predator presence, they are exposed to different a.s. concentrations over time in the short, medium and long term, ranging from hours to the whole lifespan of the organism. Organisms dwelling in a specific soil layer also experience a temporal gradient of exposure towards the active substance as it dissipates, degrades, is being sorbed and desorbed to the soil matrix or leached deeper in the soil profile through time.

7.10.3.1. Temporal field exposure profiles

In-soil organisms face exposure profiles varying over time. Since concentrations vary over time, it could be considered whether an average exposure concentration (e.g. time weighted average, PEC_{TWA}) might give a good descriptor of observed ecotoxicological effects in the test or not. Then, laboratory toxicity endpoints may be compared to average field exposure concentration (EFSA PPR Panel, 2013). In order to apply this concept, several preconditions need to be fulfilled:

- First, observed toxic effects should directly depend on the product of concentration (intensity) and time (duration). This so-called reciprocity concept (Giesy and Graney, 1989) would apply if the toxic outcome in organisms exposed to higher concentrations for short time periods would be similar to the effects observed in organisms exposed to lower concentrations but for longer time spans.
  → Some experimental results with aquatic organisms show a good prediction of chronic effects based on time-weighted average concentrations when extrapolated to other exposure regimes resulting in similar mean concentrations (same 'area under the curve' as integrated exposure concentration). Several study results, however, do not support linear reciprocity assumptions, showing that higher pulsed exposure to an active substance has stronger effects than longer exposure to lower concentrations of the same substance Please refer to EFSA PPR Panel (2013) for references to specific studies.
  → For in-soil organisms, results of ecotoxicological tests with different exposure regimes varying concentration and time span by keeping the same integrated exposure 'area under the curve' are not known.
- Second, the determination of time to onset of effects (TOE) should be possible, since knowledge of this time span determines the chosen time window over which concentrations are averaged.
  → In tests with in-soil organisms, TOE are commonly not determined. For measurement endpoints like reproduction, studies with differently timed but constant exposure pulsed would be required.

Averaged concentrations over time should *not* be used when the following conditions apply (adapted with specific reference to in-soil organisms from EFSA PPR Panel (2013); Brock et al. (2010)):

- In the assessment of chronic risk, when effect concentrations are to be used resulting from a) tests in which the exposure has not been maintained over time, b) concentration decline of the a.s. in the test system was relatively fast and c) the toxicity estimate has been expressed in terms of nominal or initially measured concentration.
  → These conditions apply almost consistently to all endpoints determined in tests with soil organism, since a) the concentrations are not kept constant throughout the test duration, b) the a.s. might adsorb to different extent to the soil matrix and c) toxicity estimates are often expressed as nominal concentration and – if occasionally measured – to initial concentrations.
- When the effect endpoint in the chronic test is based on a developmental process during a specific-sensitive life-cycle stage and evidence exists that the exposure may occur when the sensitive stage is present
  → For soil fauna, knowledge on sensitive life stages for specific developmental processes is scarce. Exposing juveniles or young adults in test systems designed to detect effects on chronic endpoints (e.g. reproduction, see Section 9) results most likely in the exposure of sensitive life stages.
- When the effect endpoint in the chronic test is based on mortality occurring early in the test (e.g. in the first 96 h)
  → does not apply frequently in the risk assessment for in-soil organisms, since current data required for the assessment of effects of active substances or PPP at lower tier are chronic data on reproductive performance. In higher tier studies with (severalfold) intended rates applied, however, this might occur.
- If latency of effects (delayed effects) occurs, resulting from delays in the chain of events between exposure and expression of effects.
  → In experiment with in-soil organisms, the observation of effects at different time intervals (and even beyond the exposure time in a clean environment) to demonstrate that latency does not occur are not performed. Delayed effects resulting from, e.g. initial high concentrations cannot be ruled out.

It is concluded from the above that further research and development of test protocols with in-soil organisms is needed to assess whether the underlying assumptions for the comparison of ecotoxicological endpoints to time-weighted average field concentrations might be fulfilled. The ecotoxicological endpoint estimates are expressed in geometric mean concentrations during the test in the EFSA PPR Panel, 2013, 2015b. In the case the preconditions are met, the use of the PEC_{twa} might be an option. If the ecotoxicological endpoint is based on initial measured or nominal concentrations, however, a PEC_{twa} should never be used in the risk assessment.

Effect concentrations for in-soil organisms that have been determined in tests with currently standard set-ups do virtually exclude the use of time-weighted average concentrations to describe the exposure that has elicited the observed endpoints. Therefore, maximum concentrations in the relevant matrix (see Table 23) are suggested as best first descriptors for acute and chronic toxic effects elicited in in-soil organisms exposed to active substances or formulated PPP. For very mobile organisms with a depth profile reaching deeper in the soil, the temporal and spatial exposure profiles might be jointly addressed in exposure models that predict degradation, dissipation and movement of the active substance and deliver spatially explicit concentrations profiles over time (please see Section 7.10.3.2 below and Section 7.11). In order to compare the outcome of different tests – especially laboratory tests with field tests – the comparability of the time course of the concentrations in the different soils is essential. However, degradation, dissipation and movement of active substance in the field soils will differ from the degradation/dissipation in a standard artificial soil (please refer to Section 7.11).

7.10.3.2. Spatial exposure profiles

Vertical distribution of the active substance

The highest concentration of an active substance that is sprayed and/or reaches the soil via drift or run-off from the treated field is generally found shortly after application and in the uppermost soil or litter layer. For PPPs that enter the soil as granules or on treated seeds or via dripping in deeper soil profiles, highest concentrations are to be found in the relevant application depth (see European Commission, 2016).

Several field and semifield studies in which fate and behaviour of PPP active substances were tightly traced over a season or a year at very high spatial resolution have been reviewed. These studies provide vertical concentration gradients of the compounds in soil horizons of one or few centimetre thickness (Fent et al., 1999; Anderson et al., 2010; Poßberg et al., 2014; Toschki et al., 2014, 2015; Egerer et al., 2015). The distributions in the soil profile of substances with different physicochemical properties and from different experiments are depicted by way of example in Figure 17. The findings of studies available with very high vertical sample resolution over time corroborate the statement that, immediately after entering the soil compartment, the active substance is located with maximum concentrations in the upper soil centimetre (or less), independently of substance properties. It should hereby be noted that due to the irregularity of the soil surface, it may be difficult to exactly determine the position of, e.g. the top centimetre of the soil, although it is possible and has been successfully achieved (see examples below). The vertical pattern with the highest measured concentration in the uppermost layer may persist over a certain time, despite differences in substances properties (e.g. Boesten, 1986; Fent et al., 1999; Evans, 2003; Poßberg et al. 2014). The influence of both degradation and sorption properties of the a.s. on the attenuation of the vertical stratification will increase with time.

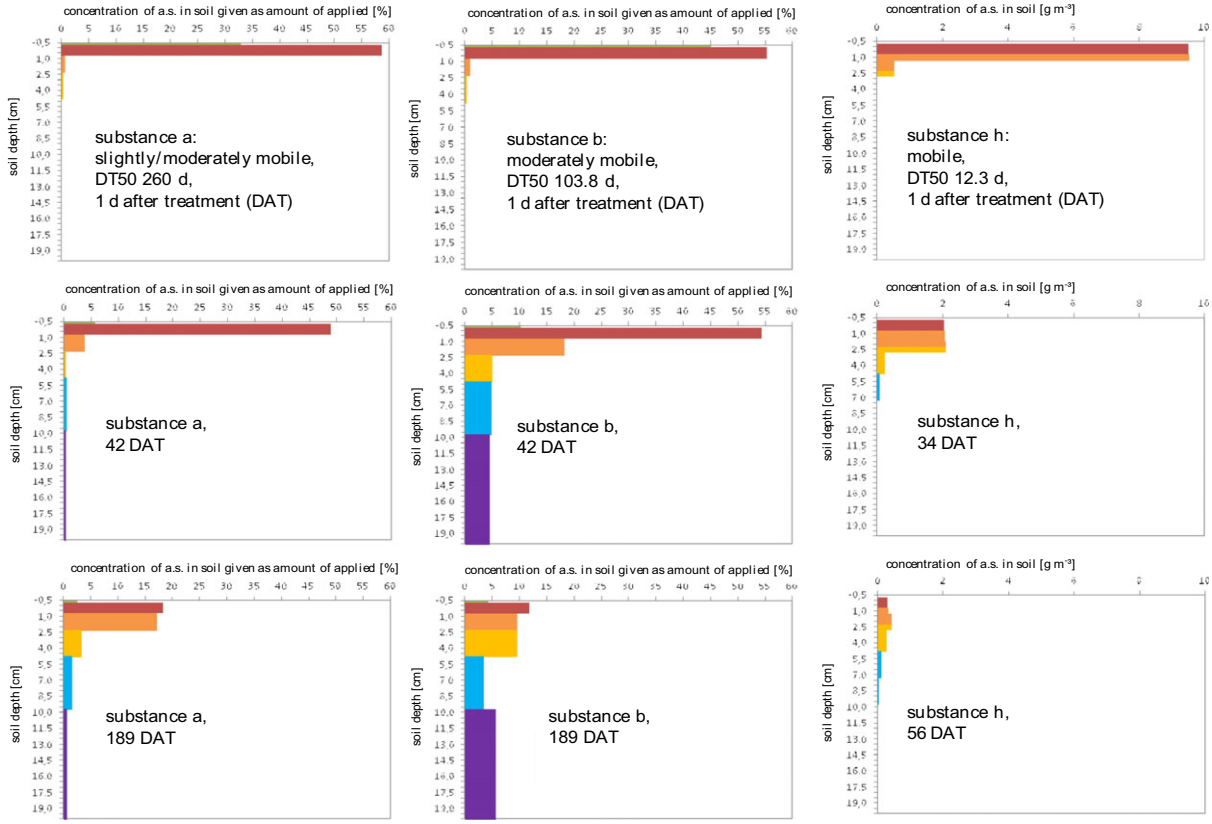

Figure 17: Distribution of three different active substances (a, b, h) in the soil profile over time (modified according to Boesten, 1986; Poßberg et al., 2014; Toschki et al., 2015; Egerer et al., 2015). The different coloured bars indicate the concentrations of a.s. in the different sampled soil depths (Copyright permissions UBA, Germany).

For further details on the distribution of active substances in the soil profile, see also Egerer et al. (2015). If the concentration in the upper soil layer is high enough to elicit acute or chronic effects, a relevant share of the in-soil organisms' community will be exposed to it, either because they live principally in the upper centimetres or because of vertical movement of in-soil organisms or a combination of both. In the field, lateral and/or vertical transport of contaminated water might also occur. If the concentration in the upper soil layer is so low that only a longer permanence of the organisms there would let internal concentrations reach levels with adverse outcome, then the behaviour and the individual spatial range of the organisms in the soil profile would be of importance for the expression of toxicity. The following paragraph will clarify these issues.

Functional groups and vertical distribution

Vertical gradients of matter and energy are manifold in the soil compartments. Nutrients, light and water are unevenly distributed. Often, these gradients correlate, with high input channels entering the soil from the soil surface, also from above-ground ecosystem compartments. In-soil organisms are adapted to these environments, shape it with their own activity and coexist via niche differentiation in strongly structured space with unexpected high species diversity. Specific adaptations to the different environments in the soil profile have led to different, more or less typical, life-form types, which are often roughly categorised as above-ground/surface dwellers (epigeic/epiedaphic species), mixed surface and soil dwellers (hemiedaphic species), soil dwellers (endogeic/euedaphic species) and 'migratory' species (anecic worms) that feed on the soil surface and dwell in the soil. These categories are however only indicative. They describe typical trait assemblages and preferences for some soil environments, but do not imply that species belonging to different groups as defined above will be only to be found in their 'preferred' soil layer, if any is known.

For microarthropods, species with different life-form traits can be found more or less stratified in soil, depending on the degree of pronounced differences between soil horizons. But even in soil profiles with a distinct litter layer, soil and litter-dwelling species change their mean depth during different seasons. Also, the distribution of individuals belonging to one species between different depths ('depth deviation' *sensu* Usher, 1970) will be narrow in some seasons and more even in others, reaching over the whole accessible profile. Usher (1970) has investigated the seasonal and vertical distribution of Collembola species and found numerous combinations of species with different mean depths and depth deviations. These distributions will change with seasons and change also during lifetime, since juveniles show distinct pattern compared to adults. In contrast to Usher (1970), Detsis (2000) analysed the vertical distribution of collembolan in southern climates. Also, here the majority of animals was found in the upper soil layer when the climatic conditions were favourable. In dry summer periods, a vertical migration of all species was observed, and 'only minute differences, if any, were observed in the vertical distribution pattern of the most abundant species, irrespectively of the life form they belong to' (Detsis, 2000). Similar patterns have been observed for oribatid mites (e.g. Mitchell, 1978), where different species were predominant in different (micro)horizons. Detailed analyses showed also for this group that unique and also more even vertical distribution patterns varied in time. As for Collembola, juvenile mites have vertical distributions that are different from the adult forms: being more vulnerable, the juveniles react more strongly and therefore earlier in the different seasons to unfavourable, but also to favourable conditions.

Even though specialised life forms might therefore typify successive layers, there is a considerable vertical migration in most soils (e.g. Lange et al., 2012). Next to the yearly phenology of species' distribution patterns, short-term changes in critical factors such as moisture regime or food supply can also initiate rather quick vertical migration.

Berthet (1964) and Wallwork (1970) found that so-called hemiedaphic oribatid species would regularly move into and from the epigeic zone, driven by the actual humidity fluctuations. One of the most striking migrations patterns in soils is shown by desert organisms (Wallwork, 1970; Whitford et al., 1980), which display an ephemeral burst of surface activity in the dew-moist morning hours, contributing significantly to litter degradation in the dry season, and then within 1 h are back again in deeper soil layers. Such vertical migratory movements have been experimentally induced by drying-rewetting experiments with, e.g. oribatid mites (Metz, 1971).

Hassall et al. (1986) showed experimentally that these vertical migrations are not only a consequence of a better accessibility of former unfavourable environments but are directed movements, induced by new food sources. In this work, more than 30% of the population of the studied onychiurid collembola moved to the very top surface within half a day – but only if palatable food was offered. According to several authors, vertical migration is a way of maintaining a balance between the possible higher mortality in upper layers (drought, predation) and reduced reproductive output resulting from less favourable feeding conditions in the lower layers (Bengtsson et al., 1991). This trade-off might even attract microarthropods of deeper layers to light when becoming increasingly starved (Dromph, 2003).

Summarising, changes in humidity and temperature alter the vertical distribution of soil animal species (Krab et al., 2010, 2015), also in the very short term, often independently from defined life form types. The ecological plasticity can be used by species to respond to soil conditions (Edwards, 2004). The needs of in-soil organisms and their perception of the soil matrix will change during their lifetime. While, e.g. neonates macrofauna might depend on water films and have a restricted mobility to existing pores, their role in shaping the soil environment will change with age (Demon and

Eijsackers, 1985). Therefore, vertical movements especially directed to the soil upper layers should be accounted for when characterising the ecotoxicologically relevant type of exposure concentration for in-soil organisms.

Functional groups of in-soil organisms in agricultural fields

The soil biocoenosis of treated areas in agricultural fields resembles for a variety of faunal groups a more or less impoverished grassland biocoenosis in terms of species diversity and individual densities (e.g. Römbke et al., 2010, 2012). Given distressing environmental conditions at the soil surface over several seasons (e.g. drought in some Mediterranean areas), anecic oligochaete key drivers might not be present or be replaced by more robust organisms (EFSA PPR Panel, 2010d). In general, however, the upper centimetres of the soil represent the principal habitat for invertebrate organisms in agricultural fields. Römbke et al. (2010) have analysed the dominance of different earthworm ecological groups in grassland and crop sites in Central Europe, reporting a very similar relative group distribution. For collembola, species numbers and dominance of different ecological groups in grasslands and in crops are reported in Table 20. Epigeic and euedaphic species show comparable shares in the in-soil organisms' community of arable and grassland sites. Also, Enchytraeids in agricultural fields might display similar but species-impoverished communities compared with grassland sites (Figure 18).

In summary, unless environmental conditions are very harsh, which may apply only to a relatively small area in Europe that is devoted to agriculture, and/or to a seasonal phenomenon, epigeic, anecic and hemiedaphic species are regularly present in agricultural fields, inhabiting the uppermost centimetres of the soil profile as well as microorganisms and fulfilling important ecosystem services (see Section 5).

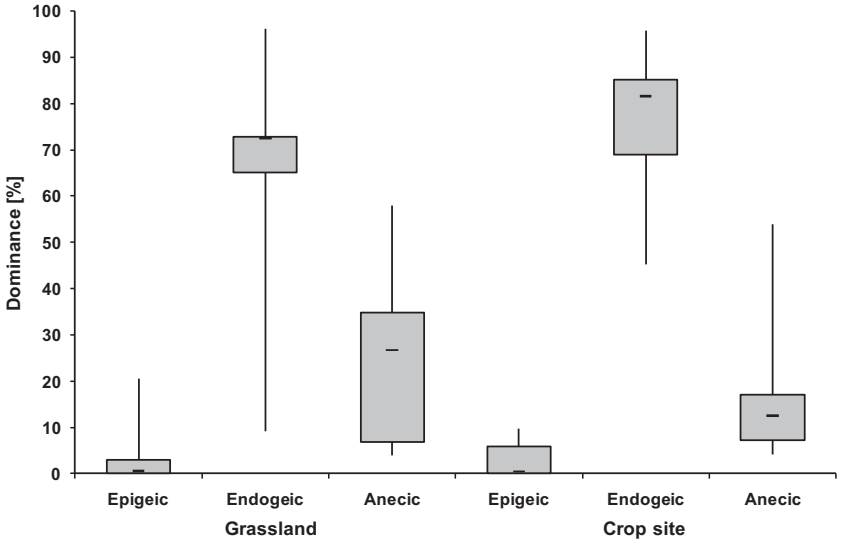

Figure 18: Dominance of the three ecological groups of earthworms at grassland and crop sites in Central Europe (Römbke et al., 2010, copyright permissions UBA, Germany)

Table 22: Average values (and limits) for the number and percentage of Collembola species in each life-form class for crop and grassland areas at Central European sites (adapted from Römbke et al., 2010)

	Epigeic species fast dispersal species, living in soil surface	**Hemiedaphic species medium dispersal species, living down to 2.5 cm layer**	**Euedaphic species species with very low dispersal ability, living down to 5 cm layer**
Crop areas (N = 12)	5 (1–14)	11 (0–24)	13 (1–29)
Species (N)	24 (9–50)	34 (0–67)	42 (17–57)
Species (%)			
Grasslands (N = 8)	5 (0–15)	8 (2–16)	11 (4–24)
Species (N)	15 (0–31)	34 (24–50)	51 (44–71)
Species (%)			

Vertical spatial heterogeneity and toxicity

As shown in the previous paragraph, in-soil organisms for which a preference for slightly deeper soil horizons is known (e.g. endogeic worms, euedaphic collembolan) might also be exposed to active substances located in the upper soil centimetres, resulting from vertical movements in the soil profile, e.g. in the search of food or moisture after raining events.

The movements of endogeic earthworms are shown by way of example in the Figure 19 below (from Capowiez et al., 2006; *Allolobophora icterica*, b). While the anecic earthworm species *Aporrectodea nocturna* (a) burrows permanent tunnels that often open to the soil surface, the endogeic species (b) processes a wider amount of soil in shallow and deeper layers. With increasing PPP concentration, the activity decreases and reduces to the topsoil.

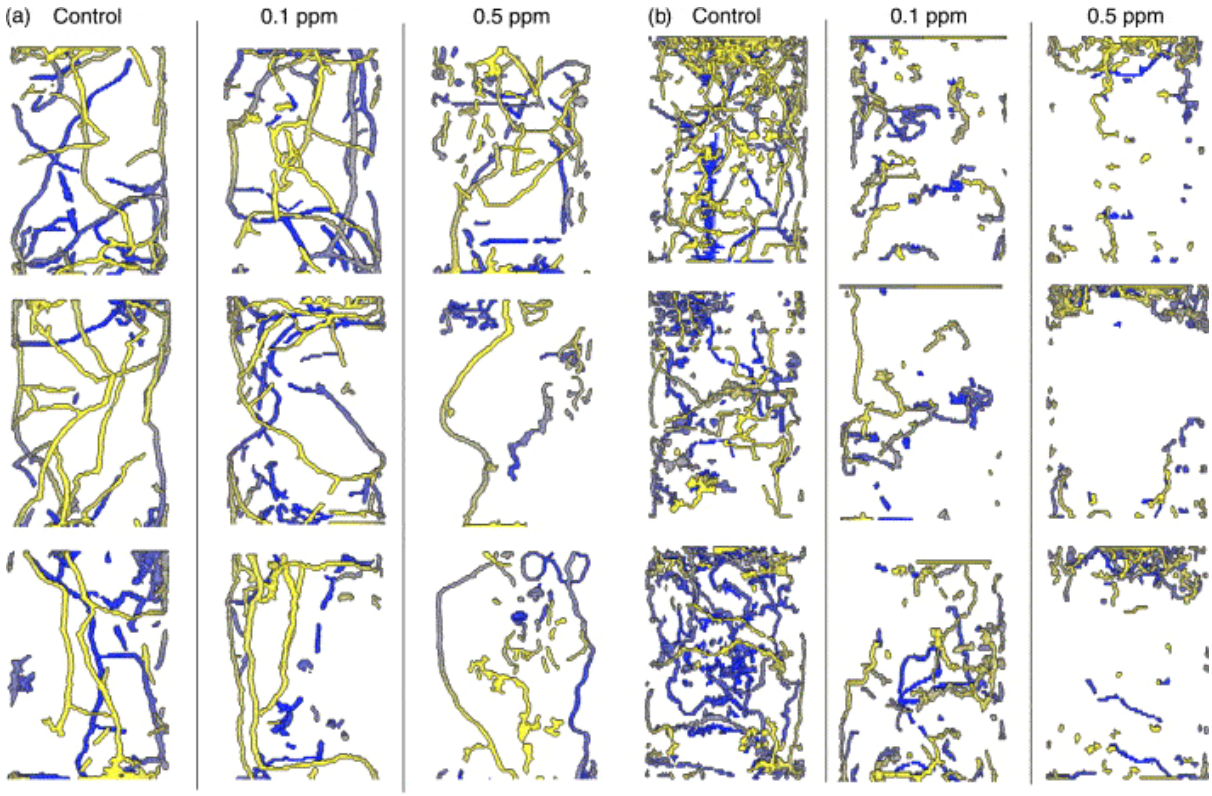

Figure 19: 3D-Reconstructions of the burrow systems made different earthworm species and increasing imidacloprid concentrations. Colours range from light to dark according to the distance from the point of observation. (a) *Aporrectodea nocturna*, anecic and (b) *Allolobophora icterica*, endogeic. Reprinted From Capowiez et al., 2006, Copyright (2006) with permission from Elsevier

Differences in behaviour between species (e.g. the amount of burrowing) may affect exposure to soil-incorporated chemicals and surface foraging to surface applications (Curl et al. 1987, 1992). The endogeic *Aporrectodea caliginosa* and the anecic *Lumbricus terrestris* were exposed to the active substance cypermethrin incorporated in the soil. Bioconcentration factors for *A. caliginosa* and *L. terrestris* were x30 and x8, respectively. This pattern is consistent with their behaviour, since *L. terrestris* has permanent burrows and forages on the soil surface, while *A. caliginosa* burrows through the soil when condition are suitable and 'digests' the ingested soil matrix.

Such observations in the laboratory were also supported by several field studies. The effects of a strobilurin fungicide, which is highly toxic to earthworm, illustrate especially the importance of considering both the distribution of the toxic compound in the soil profile and the behaviour of different species. The compound is located after spraying in the first centimetre of the soil and persists there for a considerable time without vertical movement (Evans, 2003 analytical report). On the one hand, the mean compound concentration for the investigated substance calculated for a soil-horizon thickness of 5 cm was by far not sufficiently high to explain the observed mortality effects on earthworms. Exposure to the active substance and effects on soil organisms matched only if smaller

horizon increments (1 cm) were considered for the exposure calculation (Evans, 2003). On the other hand, high mortalities of *L. terrestris* and of juvenile worms of several species were detected in the short term after irrigation, when the earthworms came to the soil surface. In the mid-term, however, the endogeic worm *A. caliginosa* showed the highest effects after a few weeks (see also Appendix E)

In an experiment with the insecticide dimethoate, Krogh (1995) set up a series of microcosms with different structured microhabitat and food-supply regimes for the collembolan *Folsomia candida*. Two of the six variants are of special interest in this context, for they addressed the question of whether an uncontaminated soil layer beneath a contaminated one reduces the observed effects on collembolan reproduction. The toxic effects of the active substance were almost identical in the two variants, leading the author to the conclusion that the collembolan species is not able to avoid dimethoate and that '[...] *F. candida* prefers eating in a contaminated soil instead of starving in an uncontaminated zone without food'.

Prinzing et al. (2002) investigated whether tolerance of disturbance in oribatid mites correlates with species traits. Two of the hypotheses tested, which stated that high tolerance to a single application of diflubenzuron was to be expected in species (a) 'with short generation time, because they can recover quickly after the disturbance'; and (b) 'which feed on fresh macrophyte detritus, because it was less altered by the disturbance than fungal microphytes', were corroborated in the study. A third hypothesis, stating that high tolerance would be found in species (3) 'which prefer the topsoil because they are less exposed to the disturbance than species that prefer the litter layer' could not be confirmed. Contrary to expectations, also of Van Straalen and Løkke (1997), the disturbance tolerance of species did not correlate with their preference for the soil layer, even if the total diflubenzuron concentrations in the soil layer was 60 times lower than in the litter.

The experiments above illustrate the difficulties that in-soil organisms might encounter when it comes to avoiding exposure to an applied active substance. Pelosi et al. (2014, and references therein) describe the usefulness of avoidance responses of in-soil organisms to contaminated soil, which can be tested according to agreed standards (ISO 17512-1, 2008). There are, however, several examples demonstrating that this ability should not be taken as given and might be specific for organisms/substance combinations (e.g. Krogh, 1995; Hodge et al., 2000; Prinzing et al., 2002).

In Scholz-Starke (2016), an evaluation of chronic studies submitted to authorities for product authorisation performed with *Eisenia fetida* and PPPs in artificial soil is presented. The working hypothesis was that mixing the substance to be assessed into the soil would lead to significantly lower effect concentrations than applying it on the soil surface, since the earthworms would dwell in the contaminated matrix. Interestingly, when substances were not specifically differentiated, test designs with sprayed chemicals onto the soil surface delivered significantly lower effect concentrations than the tests with substances mixed into the soil. When pairs of tests with the same substance were compared with each other, then the effect concentrations were similar or lower in the test variants with sprayed application compared to mixed application. Since the test protocol requests weekly feeding of the earthworms on the soil surface, the animals in the test system with sprayed substances were forced to pass through the uppermost soil layer, which contained the highest proportion of active substance.

In semifield terrestrial model ecosystems (TME), Toschki et al. (2014, 2015) investigated the distribution of three active substances with different properties (imidacloprid, lindane and carbendazim) applied to replicate soil monoliths in two different concentrations. A second set of experiments was run in the laboratory, also with soil monoliths from the same site but with ^{14}C labelled substances. Here, depth increments of 1 cm could be analysed, which was not feasible for all soil faunal samples in the outdoor mesocosms without losses in the statistical power of the assay. Analyses of the applied chemicals in high spatial resolution showed that, as expected, the largest proportion of the active substances were located in the upper soil centimetre of the soil profile during approximately the first 3 months after application.

The effects of the applied substances on earthworms, enchytraeids, Collembola and oribatid mites were also monitored over time with vertical differentiated sampling. A subset of the reported results is depicted in Figure 20. Shortly after application (14 days), effects, e.g. on earthworms in the carbendazim treatments or on Collembola in the imidacloprid treatment reached deep in the soil profile, where the respective compound could not be detected. Interestingly, organisms that are known to prefer deeper soil horizons were also affected shortly after application (for further details, please refer also to Appendix E). During the time course of the experiment, effects on in-soil organisms were found at depths where total concentrations detected were not high enough at any sampling date to explain the observed effects. The experiments were designed with dosages high enough to elicit effects on different groups of organisms with high certainty. Even if the total

concentrations detected often matched best the observed effects when related to the soil horizon thickness in which they were measured, not all comparisons of exposure and effect concentrations in the uppermost layer could be performed, since cases had to be excluded in which the concentrations were far above the medium effect concentration for short time exposure. In the TME experiments described by Toschki et al. (2014, 2015), total concentrations were assessed according to current practice and measurement of pore water concentration was not performed. The distribution of the three chemicals investigated showed pronounced vertical gradients and the information value of averaged concentrations over the soil monoliths was low compared with the actually measured total high concentrations in the top layer.

Since the observed direct effect on organism's survival result from exposure to the substances applied, the movement of soil animals towards the uppermost soil layer is most likely (see Appendix E). Some anecic worms were included in the soil monoliths and therefore biopores open to the soil surface might have been present, increasing the possibility of preferential flow reaching lower layers. Again, the preferential flow loading would be driven by the high concentrations in the upper layer and not by an averaged concentration over all depths. However, no unexpected high increase in substance residues over time was observed either in deeper soil layers or in the leachate outflow.

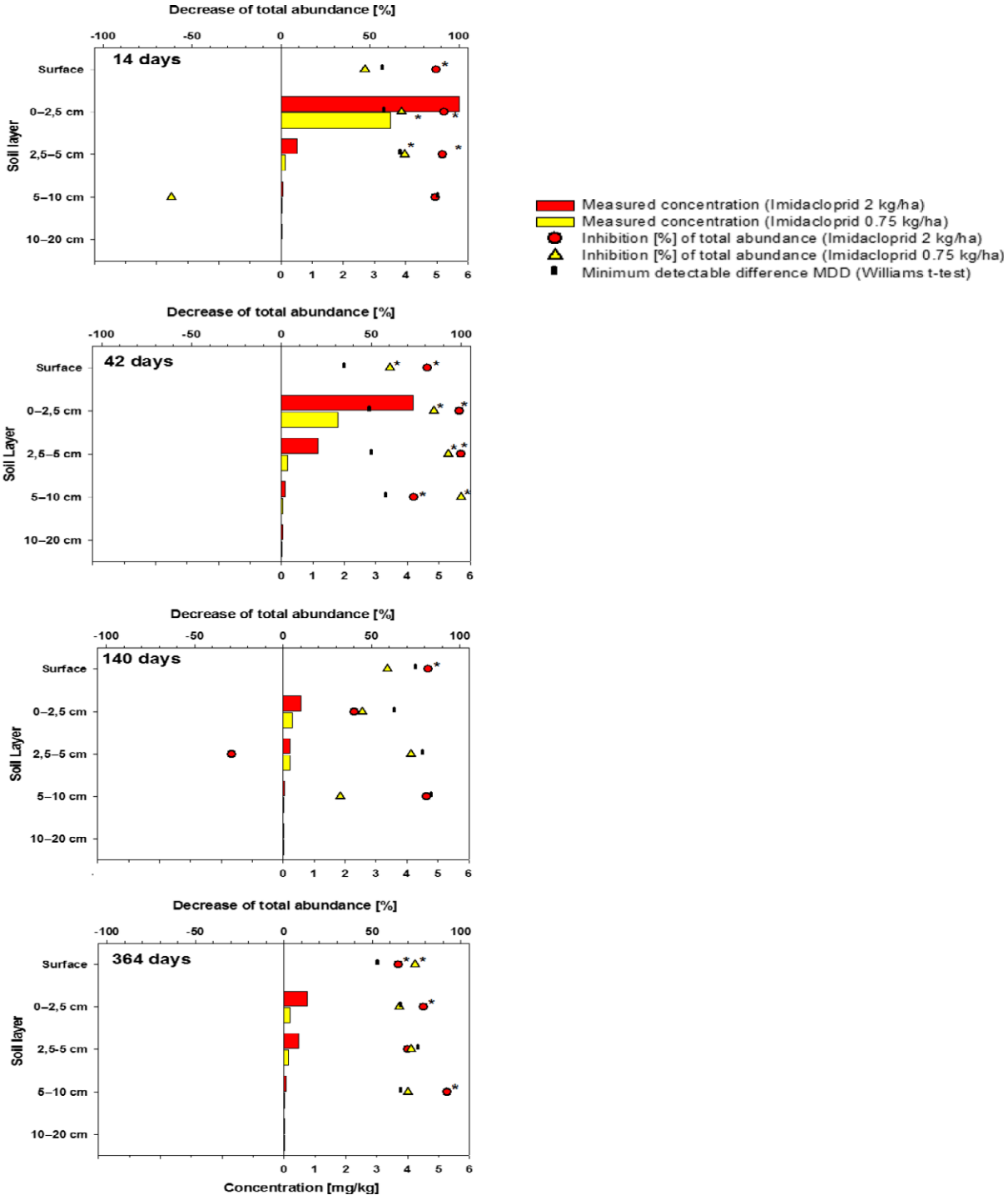

Figure 20: Decrease of total abundance of Collembolan species in the imidacloprid-treatments 0.75 kg a.s./ha and 2.0 kg a.s./ha (5 replicates each) for the different soil layers in comparison to the control (10 replicates). Columns show the measured total concentration for the two treatment concentrations at the respective sampling date. *: significant difference according to Williams t-test; bars showing the minimum detectable difference (MDD) as value for the specific possible statistical resolution. MDD values higher than 100% are not shown (from Toschki et al., 2015)

7.11. Ecotoxicologically Relevant Concentrations

Table 21 lists the major exposure routes of key drivers, specifically for each organism group. For the single exposure routes, ecotoxicologically relevant types of concentrations (ERC) are listed in Table 23 below. The proposed ERC are based on the knowledge about the major exposure routes for in-soil organisms and the considerations about the temporal and spatial exposure profiles the organisms are exposed to.

From the data referred to above, it emerges that in-soil organisms inhabiting the upper soil layer or feeding there will be exposed to the highest concentration of the applied PPP after spraying. For these organism groups, the possible uncontaminated layers below the uppermost one do not deliver a definite shelter, since other vertical gradients (e.g. of food and water) attract the animals to the surface and in-soil organisms are not able to detect and to avoid all active substances.

Averaging of exposure between high concentrations in the top layer and the low concentrations in deeper soil layers would deliver a 'space weighted average', which, as the 'time weighted average' concentration, could be used only under specific preconditions (e.g. reciprocity) and only for specific measurement endpoints (see Section 7.10.3 for details).

Organisms with a greater mobility in the soil and having a geophagous feeding mode, although, will also dwell frequently in the uppermost soil layer, but not necessarily continuously and for longer periods. If the concentrations in the uppermost soil layers are sufficiently high, then a short permanence there will also elicit acute or chronic effects. If the concentrations in the uppermost soil layer are such that only a long permanence there would lead to acute or chronic effects, then moving to uncontaminated deeper layer and feeding on uncontaminated matter would decrease the animal chemical burden. For these organisms, no single soil depth, or in many cases type of exposure, would be relevant. The choice of one, single ecotoxicologically relevant type of concentration could possibly deliver unreliable exposure metrics. To deal with this situation, exposure and effects need to be integrated over time. This could be achieved by linking following components:

- Reliable models of movement for endogeic earthworms, within the soil profile;
- Dynamic models of exposure providing soil and pore-water concentrations at all relevant soil depths and varying with time. Ideally, these would be linked to the systems models proposed for population assessment (see Section 7.3);
- Toxicokinetic–toxicodynamic models capable of integrating both internal concentrations and toxicological effects with time (see Section 9.9).

Currently, there is no available systems model combining all three components, although technically this is considered to be feasible. Ideally, this combined system would be included in the systems model used to develop the population-modelling 'surrogate reference tier' (see Section 7.3).

Since both exposure routes via total soil or pore-water concentration are considered to be relevant, and will have different relative importance for different substances and species, it is recommended to assess both exposure routes for in-soil organisms (see Table 21). Further research should clarify which exposure metrics are pertinent in different soil type/species/substance contexts.

Table 23: Ecotoxicologically relevant type of concentrations for in-soil organisms exposed to active substances in PPPs via different exposure routes.

Exposure route	Reciprocity is not demonstrated and adverse outcome can be elicited by initial concentrations	Reciprocity is demonstrated and adverse outcome due to delayed effects of initial concentrations is excluded	Adverse outcome can be related to the (time course of) internal concentrations modulated by behaviour
Contact soilmicroarthropods, nematodes, epigeic and anecic earthworms, soil gastropods, microorganisms	Maximum concentration in upper soil centimetres including accumulation (mg/kg soil dw)	Time weighted average concentration in upper soil centimetres including accumulation (mg/kg soil dw) Time frame depends on endpoint	–/–*

Exposure route	Reciprocity is not demonstrated and adverse outcome can be elicited by initial concentrations	Reciprocity is demonstrated and adverse outcome due to delayed effects of initial concentrations is excluded	Adverse outcome can be related to the (time course of) internal concentrations modulated by behaviour
Contact soilendogeic earthworms	Maximum concentration in upper soil centimetres including accumulation (mg/kg soil dw)	Time and space weighted average concentrations including accumulation (mg/kg soil dw) Time frame depends on endpoint Spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/modelled internal concentrations (mg/kg soil dw)
Contact pore watermicroarthropods, nematodes, epigeic and anecic earthworms, soil gastropods, microorganisms	Maximum concentration in upper soil centimetres including accumulation (µg/L)	Time weighted average concentration in upper soil centimetres including accumulation (µg/L) Time frame depends on endpoint	–/–
Contact pore waterendogeic earthworms	Maximum concentration in upper soil centimetres including accumulation (µg/L)	Time and space weighted average concentrations including accumulation (µg/L) Time frame depends on endpoint Spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/modelled internal concentrations (µg/L)
Oral soil organic matter microarthropods, nematodes, soil gastropods, microorganisms	Maximum initial concentration in upper soil centimetres including accumulation (mg/kg OM per soil dw)	Time weighted average concentration in upper soil centimetres including accumulation (mg/kg OM per soil dw) Time frame depends on endpoint	–/–
Oral soil organic matter endogeic earthworms	Maximum initial concentration in upper soil centimetres including accumulation (mg/kg OM per soil dw)	Time and space weighted average concentration including accumulation (mg/kg OM per soil dw) Time frame depends on endpoint Spatial resolution depends on vertical distribution of species	Spatially explicit concentrations at different time points matching the resolution of measured/modelled internal concentrations (mg/kg OM per soil dw)
Contact litter layerlitter dwelling organisms and epigeic and anecic earthworms	Maximum initial concentration in the litter layer including accumulation (mg/kg litter dw)	Time weighted average concentration in the litter layer including accumulation (mg/kg litter dw) Time frame depends on endpoint	–/–
Oral litter layer litter dwelling organisms and epigeic and anecic earthworms	Maximum initial concentration in the litter layer including accumulation (mg/kg litter dw)	Time weighted average concentration in the litter layer including accumulation (mg/kg litter dw) Time frame depends on endpoint	–/–

*–/–: The relevance of the impact of vertical movements for soil organisms living in the upper centimetres is deemed to be low.

7.11.1. Overall assessment of the exposure based on the different routes

As mentioned above, exposure by contact and oral uptake are both relevant. These two exposure routes require different exposure concentrations because the ecotoxicologically relevant concentration is different (Table 23). Developing a worst-case exposure scenario that considers both routes would require a model that integrates both exposure routes, e.g. a TK/TD model (see also EFSA PPR Panel 2014b for deriving effects based environmental scenarios). Since such models are not yet available for regulatory purposes at the European level, it is not possible to assess the relative contributions of individual exposure routes to effects. Measured effects result from the combined exposure of both routes. Extrapolation from lower tier to the field situation is done by assessment factors (Section 7.6) which address uncertainties: this includes uncertainty about the relative contributions of different routes of exposure.

Currently, available exposure data in test systems probably do not distinguish between the various exposure routes and the results are then to be seen as lumped over two routes (contact via pore water and via bulk soil and oral uptake). Available data should therefore be carefully checked and attributed to one of the two exposure routes or marked 'lumped'. Appropriate values should be used for estimating exposure according to the two specified routes (see Section 7.11.2).

7.11.2. Using consistent concentrations in the exposure and effects assessment

For the scheme in Section 7.10.1 to work, the same type of concentration should be used in the effect assessment and the exposure assessment. The type of concentration depends on the properties of the substance, the organism (e.g. soft-bodied or hard-bodied, see (EFSA, 2009c) and the intake route (Section 7.10.2) and is either the concentration in total soil or the concentration in pore water. Both types of concentrations are delivered by the exposure assessment; however, different scenarios are used for the concentration in total soil and for the concentration in pore water (Section 8).

The most commonly used tests in the effect assessment are the earthworm, collembolan and predatory mite reproduction tests (OECD 222, 232 and 226). The OECD guidelines recommend using artificial soil (70% sand, 20% kaolin clay, 10% or 5% coarse ground *Sphagnum* peat and *p*H adjusted to 6), but give also some indications to the use of natural soils. Artificial soil has the advantage that it is relatively well reproducible (Van Gestel, 1992). However, *Sphagnum* peat has different properties than organic matter in arable soils. The type of the organic matter influences sorption and hence bioavailability (EFSA PPR Panel, 2015c). Therefore, a standardised arable soil with properties closer to the scenarios in the exposure assessment would be preferred over an artificial soil with *Sphagnum* peat. However, developing a new standardised test would require extensive research to assess the suitability of the soil(s) selected to allow the survival and the reproduction of the test organisms in acceptable levels. For this reason, it is assumed that the OECD tests will be run with artificial soils in the nearest future until better alternatives are available and tested. The Panel recommends to investigate the use of feasible, alternative natural soils for standardised test systems and to perform a sensitivity analysis for a comparison of tests performed in natural soils to tests run with artificial soil.

7.11.3. Measuring exposure in test systems

In most test guidelines for in-soil organisms, the tested substance is incorporated into the soil; either in a solution or with sand. The present test systems can be adapted in order to test effects of soil fumigants, treated seeds and granules. In the past, tests were also often performed with the substance applied to the soil surface. The advantage of spraying in test systems would be that it more realistically mimics the actual exposure of sprayed substances in the field (including a layer with high concentrations in the top centimetres of soil). Nevertheless, the Panel considers mixing through the soil a better option to avoid uncertainty about the actual exposure concentration of soil organisms in the test system.

A litter layer is not included in current, first-tier laboratory tests. Exposure via the litter layer is, however, a relevant route of exposure for some soil invertebrates, like macroarthropods, slugs and snails. Furthermore, exposure via food uptake is only partly included in the standard laboratory tests, since normally uncontaminated food is provided. This may lead to underestimation of internal exposure due to dilution.

As mentioned above, either the concentration in total soil or the concentration in pore water is to be used for linking exposure and effects. Total concentrations in laboratory tests are currently commonly expressed as nominal concentrations, i.e. the total mass of chemical added to a certain mass amount of dry or wet soil. During laboratory handling procedures of the spiked soils, however, possible losses of the pesticides due to volatilisation, degradation, and sorption to, e.g. the glass matrix of vessels used, may occur. The Panel therefore recommends that the exposure concentration be measured as a function of time regardless of the metric chosen (see also (EFSA, 2009c). Measuring the concentration increases the certainty about exposure, and could deliver information about formation of metabolites (ECHA, 2016).

Exposure could be measured using the two-step extraction procedure that is proposed in EFSA PPR Panel, (2015c). This consists of a 24-h extraction with a 0.01 M $CaCl_2$ solution to characterise the pore-water concentration and a solvent extraction to characterise the total extractable mass (OECD, 2002). In principle, centrifugation would also deliver the pore-water concentration. It was, however, observed that variability was larger and therefore the $CaCl_2$ extraction is preferred. Instead of the currently used harsh solvent extraction to determine the total extractable mass, a mild organic solvent

would be preferable because this fraction generally correlates better with ecologically relevant endpoints like uptake in organisms (see Appendix 1 in EFSA, 2009c for an overview). However, no standard protocol is currently available for mild chemical extractions in relation to bioavailability testing (see also EFSA, 2009c; EFSA PPR Panel, 2015c). Furthermore, there is no commonly agreed exposure assessment methodology to determine the fraction that is available for oral uptake by organisms. The Panel therefore recommends developing (i) a protocol for characterising the fraction that is available for uptake in organisms and (ii) an appropriate exposure assessment scheme for determining this fraction. As long as these protocols are not available, it is recommended to use the concentration in total soil as a proxy for the fraction available for oral uptake by organisms.

In order to compare the outcome of different tests – especially laboratory tests with field tests – the comparability of the time course of the concentrations in the different soils is essential. However, degradation, dissipation and movement of active substance in the field soils will differ from the degradation/dissipation in a standard artificial soil. Hence, it is recommended that the concentrations of active substances in soils is measured more than twice (see also the EFSA DegT50 EFSA Guidance), depending on the degradation of the active substances and the length of the test. It should be insured that the time course of exposure in the tests covers the predicted exposure profile in the field. The time course of the exposure profile could be used in models that consider toxicokinetics/toxicodynamics (to be developed) to estimate toxicity based on internal body concentrations.

7.11.4. Calculating the exposure concentration in test systems

The Panel does not recommend calculating the pore-water concentration from the concentration in total soil using the partitioning coefficient. As indicated in EFSA PPR Panel, (2012), the coefficient for sorption on organic matter (K_{om}) is measured in at least four different soils and the variability of K_d-values between these four soils is generally more than 25%. This would give a high uncertainty of the calculated exposure concentration in the test system. However, in current ecotoxicological studies (legacy studies), only the initial nominal concentration is known. For such legacy studies, the initial pore-water concentration could be calculated when instantaneous sorption equilibrium is assumed and the water content and the sorption coefficient for the test soil are known. This can be done using the equation:

$$\frac{M_t}{V_{\text{soil}} + M_{\text{soil}} m_{\text{OM}} K_{\text{OM}}} \tag{1}$$

where c (kg/L) is the concentration in pore water, V_{soil} (L) is the total volume of liquid in the system, M_{soil} (kg) is the total mass of soil in the system and M_{t} (kg) is the total amount of substance applied, m_{om} (kg/kg) is the mass fraction of organic matter in the soil and K_{OM} (L/kg) is the coefficient of equilibrium sorption on organic matter.

The equation above is based on the assumption that sorption is linear. In reality, sorption is non-linear and because the exponent is usually < 1, the concentration in pore water will be overestimated when linear sorption is assumed. Overestimation of the pore-water concentration in the effects study would underestimate toxicity, and therefore, the Panel recommends using the Freundlich equation for calculating the pore-water concentration (in line with current practice in exposure modelling):

$$c_t = \frac{V_{\text{soil}}}{M_{\text{soil}}} c + K_{\text{om}} m_{\text{om}} c_{\text{ref}} \left(\frac{c}{c_{\text{ref}}} \right)^{1/n} \tag{2}$$

where c_{t} (mg kg) is the concentration in total soil, c_{ref} is the reference concentration (usually 1 mg/kg) and $1/n$ is the Freundlich exponent. This equation requires an iterative solution, which could be easily implemented using, e.g. standard spreadsheet software.

Notice further that the equations above only give the initial concentration of a parent substance. If a time-weighted average concentration is needed, it could be calculated assuming first-order degradation kinetics. For this purpose, the Panel recommends developing a dedicated version of the PERSAM model (EFSA PPR Panel, 2012). This tool could also be helpful for characterising the exposure concentration of metabolites in the test system. Specific attention is needed for parameterisation of this model so that it will lead to conservative estimates of the pore-water concentration (Van der Linden et al., 2006, 2008).

Time-dependent or aged sorption is a generally accepted phenomenon, which reduces the concentration in pore water (see e.g. EFSA PPR Panel, 2015c). Consideration of this process is not necessary if measured pore water concentrations are used in the effects study, because this process is then implicitly included in the measurements.

7.11.5. Scaling of the toxicity endpoint to account for bioavailability

It is now common practice to divide the obtained toxicity endpoint (LC_{50} or NOEC) of lipophilic substances ($\log(K_{ow}) > 2$) for in-soil organisms (except microorganisms) by a factor of two. This is done because the organic matter content of the artificial substrate of the earthworm laboratory tests is higher than that of many natural soils (Van Gestel, 1992). The scaling factor was needed because concentrations were only available as nominal concentrations (Section 7.11.5), while toxicity endpoints generally correlated better with pore-water concentrations. This was demonstrated because differences between soils almost completely disappeared when LC_{50} values in mg/kg were recalculated towards values in mg/l pore water concentrations (Van Gestel and Ma, 1988, 1990; see also EFSA, 2009c).

Scaling of the toxicity endpoint is no longer necessary (and even not justified) if the appropriate exposure concentration in the test system is either measured (Section 7.11.3) or calculated (Section 7.11.4). When only the nominal concentration is available (legacy studies) and when the ERC is expressed in terms of a pore-water concentration, scaling of the toxicity endpoint is justified (Table 23). Notice, however, that the factor of two has no scientific basis for assessments at the European level because it is based on the ratio of the organic matter content of the test medium (10%) and the organic matter content of the old Dutch standard soil (4.7%) and not on that of the proposed exposure scenarios described in Section 8. So, instead of the default factor of two, scaling should be done based on the following equation:

$$S_f = \frac{V_{soil} + K_{om}OM_{test}M_{soil}}{V_{soil} + K_{om}OM_{scenario}M_{soil}} \quad (3)$$

If in the equation V_{soil} is neglected, equation 3 reduces to:

$$S_f = \frac{OM_{test}}{OM_{scenario}} \quad (4)$$

Figure 21 gives the scaling factor calculated with equation 3 for a range of values of K_{om}. In this example, the mass fraction of organic matter in the test medium is 0.1 g/g (or 10%). The figure indicates that the scaling factor is substance-dependent. For substances with a $K_{om} > 10$, the equation yields the traditional factor of two when the organic matter content of the test medium is 10% and the organic matter content of the scenario is 5%. For lower values of K_{om}, ignoring V_{soil} would overestimate the scaling factor, and would therefore give a conservative estimate of the toxicity endpoint.

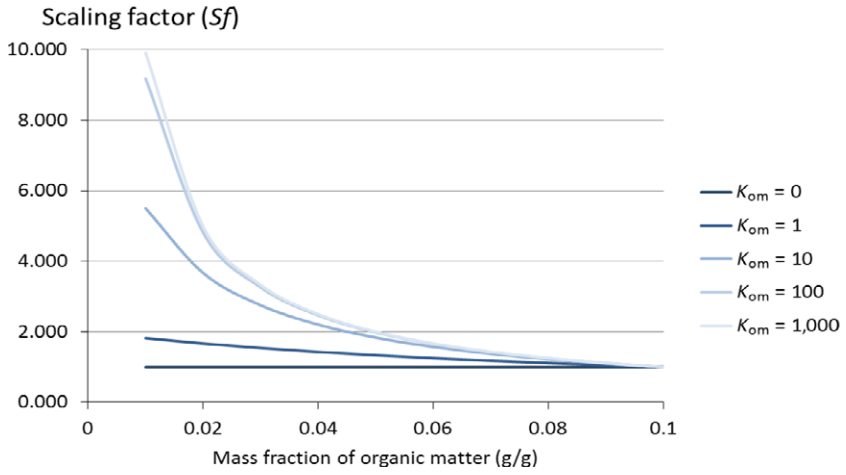

Figure 21: Scaling factor of the toxicity endpoint as a function of the mass fraction of organic matter of the exposure scenarios for a range of values of K_{om}. In this example, the mass fraction of organic matter in the test medium is 0.1 g/g (or 10%)

Table 24: Recommended scaling factor to account for differences between the concentration used in the effects study and the ecotoxicologically relevant concentration.

Type of concentration measured or calculated in the effects study	Ecotoxicologically Relevant Concentration*	Scaling factor for 3 toxicity endpoint
Concentration in total soil	Concentration in total soil***	1
Concentration in pore water	Concentration in pore water	1
Concentration in total soil	Concentration in pore water	S_f calculated with Equation 2**

*: See Section 7.11 for a description of the ecotoxicologically relevant concentration.
**: OM_{test} (%) is the organic matter content of the test medium and $OM_{scenario}$ is the organic matter content of the selected exposure scenario.
***: The Panel recommends using the concentration in total soil also when effects correlate best with a mild organic fraction instead of the total concentration, which is the case for, e.g. oral uptake.

The implicit assumption of this scaling is that organic matter is the main adsorbent (Van Gestel, 1992). The latter is true for the majority of PPPs; however, in some cases, PPPs show affinity for other soil constituents such as clay minerals or sesquioxides (see EFSA PPR Panel, 2015c) for an overview of sorption processes). Clearly, in those cases, the scaling procedure is not valid and the pore-water concentration should be calculated or measured. Van Gestel (1992) further mentions that for substances with a log(Kow) < 2 differences in soil moisture content should be taken into account when extrapolating toxicity data. However, current practice at the EU level is not to scale the toxicity endpoint at all for such substances. This is not in line with the original description given by Van Gestel (1992).

If the PEC is derived from a Tier 1 or 2A exposure assessment (see Section 8), the organic matter content of one of the standard concentrations in total soil scenarios should be selected. If the PEC is derived from a Tier 3 assessment (substance or crop specific exposure scenarios), the organic matter of this Tier 3 scenario should be used. Notice that organic matter content is not delivered when running a Tier 2B/C exposure assessment. It can, however, easily be obtained by running Tier 3B. It has to be noticed that the scaling factor is less than one when the organic matter content of the selected exposure scenario is higher than the organic matter content of the test system.

8. Exposure Assessment

8.1. Introduction

8.1.1. The EFSA Guidance for exposure assessment for in-soil organisms

In the EU, PPP exposure assessment for in-soil organisms was traditionally based on the FOCUS methodology on persistence in soil (FOCUS, 1997; European Commission, 2000). However, during a general consultation of Member States on needs for updating existing Guidance Documents and developing new ones, a number of EU Member States (MSs) requested a revision of the SANCO Guidance Document on persistence in soil (SANCO/9188VI/1997 of 12 July 2000). The consultation was conducted through the Standing Committee on the Food Chain and Animal Health.

A draft EFSA guidance (EFSA, 2016) has recently been published for public consultation laying down a new exposure assessment for plant protection products (PPPs) and their transformation products for in-soil organisms according to Regulation EC No 1107/2009 of the European Parliament and the Council. The document provides methodologies for calculating all types of concentrations that could be relevant for assessing ecotoxicological effects, including the concentrations in total soil and concentrations in pore water, both averaged over various depths and time windows.

As described in EFSA PPR Panel (2012), the methodology is based on the goal to assess the 90th percentile concentration considering all agricultural fields within a regulatory zone (North–Central–South) where the particular PPP is intended to be used. The agricultural area of use is represented by the crop in which the pesticide is intended to be used, e.g. for a pesticide that is to be applied in maize, the area is defined as all fields growing maize in a regulatory zone. By defining the total area as the regulatory zones within the EU (Figure 23), considerably fewer scenarios were distinguished here than in earlier guidance, which used climatic and pedological data to identify scenarios (e.g. Forum for Co-ordination of Pesticide Fate Models and their Use (FOCUS) Groundwater reports of 2000 and 2014, in which nine scenarios were distinguished). This was implemented to keep the regulatory process as simple as possible. In general, exposure estimates for all three zones should be evaluated

for review of substances at the EU level. For zonal evaluations of PPPs, it would be sufficient to consider only the exposure estimates for the particular zone in question.

The recommended exposure-assessment procedure consists of four tiers (EFSA, 2016). To facilitate efficient use of the tiered approach in regulatory practice, user-friendly software tools have been developed for the first three tiers. This includes the software tool PERSAM (Persistence in Soil Analytical Model) and new versions of the pesticide fate models PEARL (Pesticide Emission At Regional and Local Scales) and PELMO (Pesticide Leaching Model). The software tools generate reports that can be submitted for regulatory purposes. Users of the guidance are advised to use these software tools when performing the exposure assessment. Models other than PEARL or PELMO are currently not supported unless the process descriptions in such numerical models have a similar or higher level of detail than those in PELMO and PEARL (EFSA PPR Panel, 2012a). Furthermore, it should be demonstrated that the models give similar results to PEARL and PELMO. This is necessary to guarantee consistency of the tiered approach. According to EFSA (2016), plateau concentrations after multiyear applications are always calculated.

Although the standard output of the exposure models is a limited number of maximum and time-weighted average concentrations, the numerical models PEARL and PELMO are able to deliver concentration-time profiles in high resolutions. This additional information could be used for further modelling, for example as input into TK/TD models to estimate time-dependent, internal concentrations in the bodies of the species.

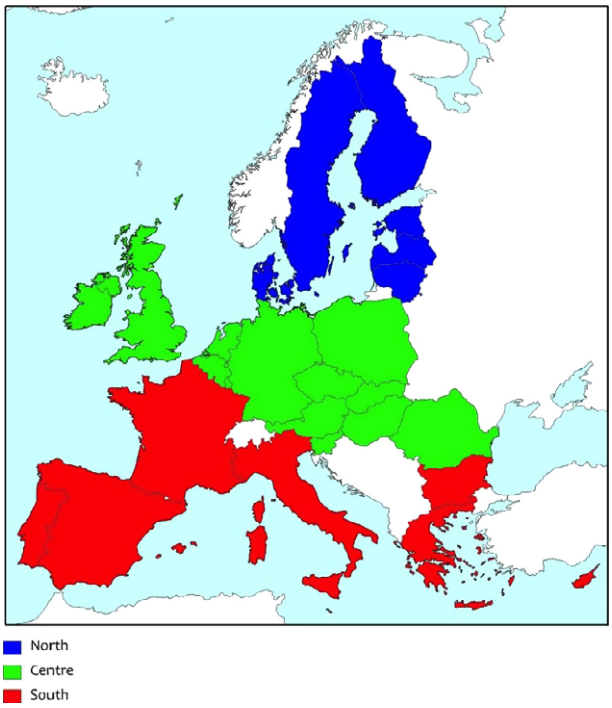

Figure 22: Map of the three regulatory zones according to Regulation EC No 1107/2009 of the European Parliament and the Council

8.1.2. In-field and off-field exposure

Off-field exposure (e.g. as a result of spray drift deposition or as a result of storage or disposal of growing media used in horticultural production) is not covered by EFSA (2016) because the proposed methodology does not describe emissions from the treated field and subsequent deposition of the emitted amounts onto the off-field surface. This chapter therefore provides also some considerations for off-field exposure; however, appropriate off-field exposure scenarios that apply to a given percentile of the concentration distribution still need to be developed.

For the definitions of the spatial scales used in the environmental risk assessment of in-soil organisms, please see Section 6.1 and Figure 9.

8.2. Overview of the tiered approach

The proposed exposure assessment starts with simulations for one predefined scenario per regulatory zone, North–Central–South. At this tier, simulations are carried out with the simple analytical model PERSAM. PERSAM has the advantage that the required number of inputs is very limited and thus the documentation will also require little effort.

Two sets of predefined scenarios have been developed, i.e. one set for annual field crops and one set for permanent crops. The predefined scenarios for annual crops in Tier-1 are based on the total area of annual crops in a regulatory zone and the predefined scenarios for permanent crops are based on the total area of permanent crops. However, the exposure assessment goal is based on the agricultural area where a PPP is intended to be used. The applicant may therefore wish to perform an exposure assessment for a particular crop. For this purpose, Tier-2 is provided. At this tier, a spatially distributed version of PERSAM is used and the target percentile is directly calculated from the concentration distribution within the area of a given crop. Should the assessment at Tier-2 still indicate an unacceptable risk to soil organisms, the applicant has the option to move to Tier-3. Tier-3 is also based on the area of a given crop, but uses numerical models (PEARL and PELMO). Tier-3A requires slightly more effort; however, this tier has the advantage that more realistic modelling approaches are used and therefore this tier will deliver less conservative values. In Tier-3A, crop-specific and substance-specific scenarios are used. Guidance is given on how to select and use these scenarios.

Tier-1 is based on the assumption that crop interception of the substance does not occur. In all other tiers, this can be included. Interception and subsequent dissipation at the crop canopy may be based on simulations with the numerical models. To facilitate harmonisation of the regulatory process, canopy processes in PEARL and PELMO were harmonised. This guidance further introduces a table for the fraction of the dose reaching the soil surface that was created based on simulations with PEARL and PELMO. This table should be used at Tier-2. More detailed information on how to perform calculations at different tiers can be found in EFSA (2016). More information on how to combine the exposure concentrations with toxicity data can be found in 7.10.1.

8.3. Cropping and applications systems covered by the new guidance

The methodology covers a wide range of different cropping and application systems (Figure 22). The exposure assessment for annual crops differs from that for permanent crops because the distribution of organic matter with depth in permanent crops differs from that in annual crops. For this reason, the exposure assessment scheme makes a distinction between annual crops (left-hand side of Figure 22) and permanent crops right-hand side of Figure 22).

The guidance does not cover all cropping and application systems. For uses that are not covered by the new EFSA guidance (for example spot treatments) (EFSA; 2016), the applicant should describe how the guidance document is implemented and justify its applicability.

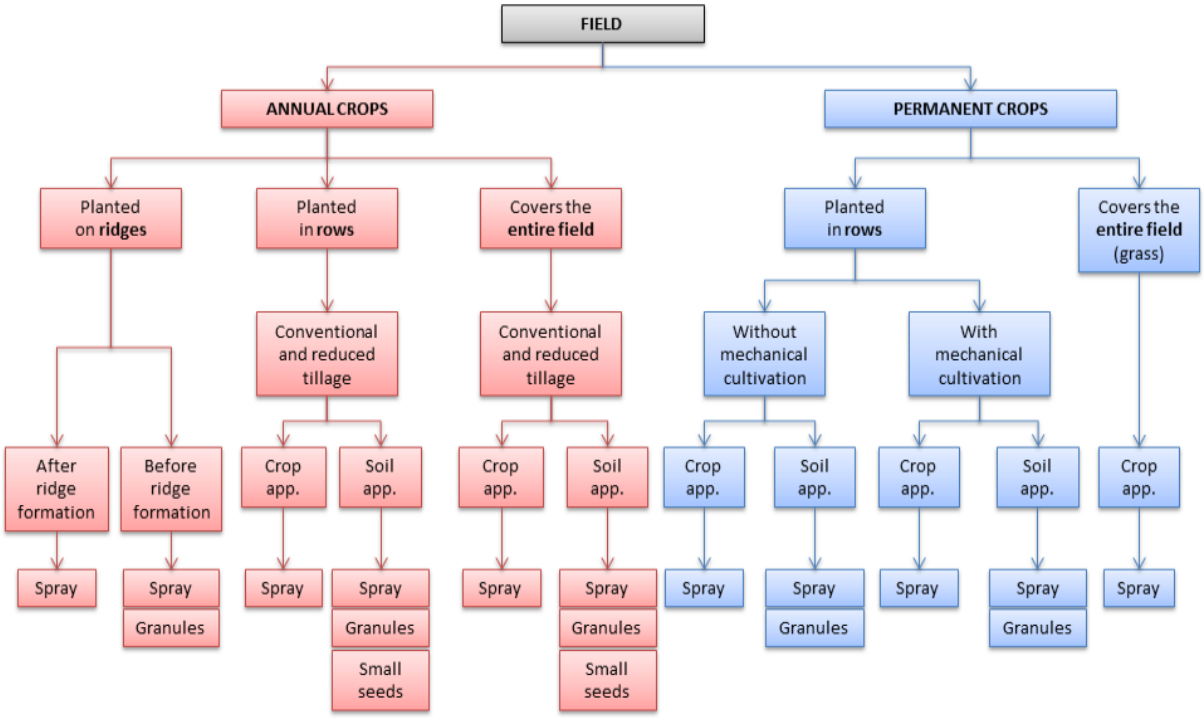

Figure 23: Cropping and application systems covered by this guidance

8.4. Litter layer

According to Beulke et al. (2015), data on the litter layer is scarce and it is assumed that no litter layer is present in the majority of the permanent crops. Litter might, however, become more important in the future as good soil-management practices are promoting the presence of organic matter on the soil, so there may be a shift to a more sustainable management of this litter layer. In addition, although there is usually no permanent litter layer in current agriculture, there may be litter debris in the field temporarily, which could be a significant exposure route for some species. Unfortunately, the exposure models used presently in registration are not able to simulate the fate of pesticide in litter and no scenarios have been defined so far to cover this exposure route. However, a simple estimation of the peak concentration in litter could be based on the current soil scenarios considering the application rate, the crop interception at the time of application, and an appropriate density of litter. Nevertheless, the development of more advanced computer models that also consider additional processes (e.g. the uptake of pesticide via the plant roots) would be necessary to describe this exposure completely.

8.5. Exposure routes in the off-field area

The presence of PPPs on off-field non-target surfaces (plants, non-target arthropods, etc.) is a combination of three processes during and after the application of the compounds in the field: (i) the emission of the applied product out of the field by spray drift and runoff, (ii) the deposition of the emitted amounts onto the off-field surfaces and (iii) dissipation processes from the non-target surface. Drift is currently considered to be the most important factor for off-field emissions to non-target surfaces. However, depending on the meteorological conditions shortly after application, losses due to surface run-off may also contribute to the contamination of non-target, terrestrial ecosystems in the neighbourhood of agricultural areas. Other emission routes such as leaching and drainage are not considered as direct emission routes. Drift is defined as droplet drift but vapour drift and dust drift are also considered to be important emissions in particular cases. 'Deposition' on non-target surfaces is defined as the entry path for transport of airborne or waterborne substances from the air to the non-target surface, i.e. to an aquatic or terrestrial compartment or directly to non-target plants, arthropods, bees, etc. Dry and wet depositions should be considered separately because they are subject to different atmospheric and physical processes.

In the absence of appropriate off-field exposure scenarios that apply to a given percentile of the concentration distribution, it is advised to base the calculation of soil concentrations in the off-field area on the scenarios for in-field exposure as described in EFSA (2016). As mentioned in EFSA PPR Panel, (2010b), the exposure estimate should preferably apply to a given percentile of the concentration distribution (usually the 90th percentile) of the treated fields. Developing an exposure scenario for a given percentile requires simulating the concentration distribution in the entire target area (e.g. EFSA, 2016). The model for simulating this concentration distribution should preferably include all relevant exposure routes (i.e. spray-drift deposition, vapour-drift deposition, dust-drift deposition and surface run-off). Since such models are not yet available for regulatory purposes at the European level, the simplifying assumption is made that the individual exposure routes can be assessed separately. Results of the different entry routes should then be summed, which is a conservative assumption because it neglects the different dynamic behaviour of the processes.

8.5.1. Spray drift/deposition

Spray drift is defined as that part of the applied product that leaves the treated field through air because of air current during the application of the plant protection product. These spray drift emissions do not include emissions by volatilisation. Droplet drift is considered to be a short distance process (0–30 m) and occurs only during and shortly after application (i.e. within a few minutes actually defined by the time between spraying and collection of samples during drift experiments).

Spray drift is not compound-specific but mainly dependent on droplet size, wind speed, wind direction and crop and spray-boom height during spraying. The spray drift is calculated on the basis of spray-drift tables, which give the deposition as a percentage of pesticide-application rate deposited at a given distance from the last crop row as a function of crop type (arable crops, fruits, grapes, hops and vegetables), the crop stage (early or late) and the spraying technique. Different spray-drift curves are available (Southcombe et al., 1997; Rautmann et al., 2001). The most recent publication on the topic is a report that presents joined spray-drift curves from German and Dutch experiments (Van de Zande et al., 2015).

However, the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge of field surface waters (EFSA PPR Panel, 2013) describes estimating the drift deposition on surface water with the spray-drift values reported by FOCUS (2001), which are based on Rautmann et al. (2001) and apply to 90th percentile conditions. Nevertheless, it could be more appropriate to base further assessments on the most recent publication by Van de Zande et al. (2015). Independent of the data set, all measurements were obtained from deposit measurements on artificial receptors (e.g. filter paper strips) on bare soil. The use of these data sets also for the off-field area can therefore be considered a worst case.

Generally, the risk assessment for the off-field area could consist of two steps. In the first step, the exposure could be based on the standard spray-drift curves in-field risk assessment, i.e. the drift deposition would be 100% of the application rate. If the protection goal for the off-field area would not be met in this step, risk-mitigation options would have to be assessed in a follow-up step. Options to mitigate spray-drift deposition to off-field areas include (i) the use of spray-drift reducing techniques and (ii) the establishment of non-spray buffer strips, with or without crop. Since spray-drift deposition decreases with both distance and drift-reducing technique class, spray-drift mitigation options could be evaluated using a matrix. Spray-drift deposition could for example first be evaluated for the standard spraying technique, second for drift-reducing techniques and measures, and third for all spray techniques with stepwise wider buffer strip.

Spray-drift deposition differs between crop types (grass and bare soil, field crops, fruit crops, vines and hops) and crop-development stage. For this reason, a spray-drift deposition curve and hence evaluation matrix is needed for each combination of crop type and crop-development stage, or classes of these. For estimating spray-drift deposition onto surface waters, spray-drift deposition curves were developed by the FOCUS Surface Water work group (FOCUS, 2001) for many major crops. Harmonised European drift curves are currently only available for bare soil, grass and fully developed arable field crops (Van de Zande et al., 2015); however, spray-drift curves for fruit crops are expected to become available in the near future. For vine and hops, no updated values are foreseen in the near future. In this situation, the PPR Panel recommends that the efficiency of new spray-drift curves is evaluated when they become available and that the spray-drift assessment methodology is revised accordingly. For the time being, the current assessment based on Step 2 in FOCUS (2001) can be used.

8.5.2. Vapour Drift

Vapour drift can occur by (1) evaporation of the solvent from small spray droplets that are still present as 'drift' after application and (2) post-application evaporation of the spray deposits from treated plant/soil surfaces. Vapour-drift deposition is usually short to medium range (0–1,000 m). Most emission by volatilisation occurs during the first 24 h after application and is caused by spray-droplet evaporation. However, evaporation from deposits may continue for several days or weeks after treatment (Bedos et al., 2002). Evaporation of the leaf/soil deposits is dependent on the active ingredient properties, such as volatility, and interaction with leaves. Volatilisation from plant surfaces is one of the main pathways of pesticide emission to the environment and normally is greater than volatilisation from soils because plants have fewer sorption sites than soil.

The main factors controlling pesticide volatilisation are the physicochemical properties of the pesticide (in particular vapour pressure), agricultural practices (time and type of application), soil or plant physical properties and meteorological conditions (during and after application). Several models for vapour-drift emissions were evaluated by the FOCUS Air working group (FOCUS, 2008). They concluded that none of the models available completely fulfilled the requirements for use within a regulatory context. For pragmatic reasons, FOCUS (2008) recommended using the EVA 2 model for calculating the deposition after volatilisation for short-range transport. Later, the PPR Panel has evaluated this model and came to the conclusion that the recommendations regarding the use of the EVA 2 model are scientifically not robust enough (EFSA, 2007). The PPR Panel further came to the conclusion that the recommended model does not give realistic worst-case exposure estimates. Therefore, the PPR Panel recommends improving the estimation of vapour-drift deposition by the EVA 2 model and also to investigate the option to use alternative modelling approaches since these have now become available. However, for the time being, the current assessment based on FOCUS (2008) can be still used for regulatory risk assessment.

FOCUS (2008) stated that volatilisation is only relevant for compounds with a vapour pressure higher than 10^{-4} Pa when applied to the soil and for compounds with a vapour pressure higher than 10^{-5} Pa when applied to the crop. In this context, it is worth noting that, whereas it is possible to minimise droplet-drift emission to the off-field area using appropriate application techniques (e.g. drift-reducing nozzles, buffer zones), this does not apply to volatilisation, since this process is mainly driven by pesticide and crop properties. The relative contribution of vapour-drift deposition is demonstrated below. Table 25 and Table 26 summarise calculations with the EVA 2 model for a compound with medium volatility (vapour pressure of 5×10^{-3} Pa) assuming various crop-interception fractions in the field. The consideration of crop interception is necessary, since volatilisation from the crop canopy is estimated to be three times higher than volatilisation from the soil surface. For the crop-interception values, the most recent numbers are recommended (EFSA PPR Panel 2014a).

Table 25: Spray drift and volatilisation deposits for arable field crops calculated with EVA 2*

Distance (m)**	Droplet Drift (µg a.s./m²)	Cumulative volatilisation deposits over 24 h (µg a.s./m²) dependent on crop interception in field			
		No interception	25% interception	70% interception	90% interception
1	2,770	518	778	1,244	1,451
3	943	465	697	1,116	1,302
5	570	417	625	1,000	1,167
10	290	318	476	762	889
15	200	242	363	580	677
20	150	184	276	442	516
30	100	107	160	256	299
50	60	36	54	86	101
100	30	2	4	6	7

*: Application dose 1 kg/ha, vapour pressure 5×10^{-3} Pa.
**: From last row of treated crop.

Table 26: Spray drift and volatilisation deposits in orchards (early) calculated with EVA 2*

Distance (m)**	Droplet Drift (µg/m²)	Cumulative volatilisation deposits over 24 h (µg/m²) dependent on crop interception in field			
		No interception	25% interception	70% interception	90% interception
1	–	1,814	534	548	670
3	29,200	1,627	479	492	601
5	19,890	1,459	429	441	539
10	11,810	1,111	327	336	410
15	5,550	846	249	256	313
20	2,770	645	190	195	238
30	1,040	374	110	113	138
50	300	126	37	38	46
100	60	8	2	2	3

*: Application dose 1 kg/ha, vapour pressure 5×10^{-3} Pa.
**: From last row of treated crop.

It should be noted that the depositions of vapour drift in the tables are the cumulative exposure over 24 h. They are compared with the deposition of spray drift, which can be considered as an instantaneous event actually defined by the time between spraying and collection of samples during drift experiments, usually 15 min. Further information about the equations in the model is given in EFSA PPR Panel, (2014a).

If the deposition rate exceeds the maximum acceptable dose, risk assessors might want to establish a buffer strip. Similar to the calculation of drift deposits, EVA does not directly allow for the calculation of the width of buffer strip necessary to meet maximum acceptable deposits. However, this is possible when transforming the original equation as described in EFSA PPR Panel (2014a).

The next table (Table 25 and Table 26) shows examples for different volatility classes when 1 kg/ha was sprayed and the maximum acceptable load was calculated to be 0.01 kg/ha.

It has to be noted that the numbers in Table 25 and Table 26 are based on a very volatile compound, while the numbers in Table 27 are based on a range of substance with different volatility.

Necessary buffers (m) to prevent non-acceptable volatilisation deposits*

Vapour pressure range at 20°C	Relative volatilisation from canopy, deposit at 1 m (% of application dose)	Necessary distance (m) no interception (in field crop)	Necessary distance (m) 100% interception (in field crop)
$vp < 10^{-5}$ Pa (plant) $vp < 10^{-4}$ Pa (soil)	0.00	No buffer	No buffer
10^{-4} Pa > vp > 10^{-5} Pa	0.09	No buffer	No buffer
5×10^{-3} Pa > vp > = 10^{-4} Pa	0.22	No buffer	No buffer
vp > = 5×10^{-3} Pa	1.56	No buffer	9.17

*: Application dose 1 kg/ha, maximum acceptable load: 0.01 kg/ha.

In the table, a buffer zone was calculated only for the compounds having vapour pressures above 5×10^{-3} Pa and when the application was targeted fully at the (target) canopy. That demonstrates that in most situations deposition caused by droplet drift will be the dominant entry route rather than volatilisation deposits.

8.5.3. Particulate drift

Particulate drift can occur due to (1) application of dust from dustable powder formulations (e.g. sulfur dusting in vineyards), (2) dust formation during non-spray applications (NSA), e.g. granules (fertiliser–herbicide combinations for application in lawns) and treated seeds, or (3) soil dust with adsorbed pesticide deposits. However, the latter emission is not considered to be a direct emission route.

Particulate drift happens generally over a short range and in short periods after application and is thus comparable to droplet drift. The main driving force is the particle size/weight of the dust particles.

The EFSA Opinion on non-spray applications (EFSA, 2005) gives guidance for the exposure assessment of NSAs (non-spray applications). The main conclusions and recommendations for dust drift are:

1) Dust in NSA is a relevant route of exposure for surface water.
2) Broadcast granular applications even with subsequent incorporation can form dust drift that can have comparable effects as spray drift.
3) Abrasion dust of treated seeds generated during broadcast application is also considered to be a relevant route of exposure.

According to the EFSA Opinion on non-spray applications (EFSA, 2005), dust drift can be handled by FOCUS surface-water models with adjustment of the normal default inputs in such a way that an evaluated dust-drift value is entered. For default values, adapted spray-drift models can be used to estimate dry deposition from dust by taking into account a number of specific, underlying criteria.

As there is currently an increasing concern with regard to dust drift and seed treatments, the European Commission recently prepared a document that includes experimental data from dust-drift deposition for different crops (European Commission, 2016). There are, however, still problems remaining when considering the experimental studies on dust-drift deposition in the document by the European Commission (European Commission, 2016) in the same way as the current spray drift numbers, as there is not a direct link to the application rate. Instead, the sowing rate has to be calculated, first, which may be expressed in number of seeds per ha or in kg of seeds per ha depending on the crop or the region which is based on. Furthermore, in contrast to the standard FOCUS drift values, the evaluation performed in EC (2016) concentrates on very short distances of the off-crop area (i.e. 1 m) which makes it difficult to define safe areas over longer distances.

However, seed treatment quality can be nevertheless improved by certification.

8.5.4. Run-off entries

This section deals with the assessment of pesticide movement to surface water caused by run-off and its links to the terrestrial compartment. The assessment of pesticide movement to surface water caused by run-off is currently a key process in European risk assessment. The recommended methodology as described by FOCUS (2001) follows a tiered approach. Run-off occurs after heavy rainfall events, which may transport residues of the active substance or transformation products either dissolved in the water or sorbed to the eroded sediment particles to the non-target area. If mitigation measures for run-off entries reaching surface waters are used (e.g. vegetated buffer strips), this could be connected with deposition of residues to the terrestrial ecosystems. However, there is currently no regulation indicating if a vegetative buffer strip is to be considered as an off-crop area or not.

For the estimation of run-off and erosion losses leaving the edge of field, several models are available, e.g. the models used in the different tiers of FOCUS surface water (FOCUS, 2001). At tier II, pesticide losses by run-off as summarised in Table 28 are considered.

Table 27: Step 2: pesticide losses by run-off and soil erosion according to FOCUS STEP 2

Region/season	% of soil residue
North/Centre* Europe, October–February	5
North/Centre* Europe, March–May	2
North/Centre* Europe, June–September	2
South Europe, October–February	4
South Europe, March–May	4
South Europe, June–September	3

*: According to FOCUS (2001), the number also reflects the situation in Northern France.

For pragmatic reasons, the losses due to run-off at step 2 were defined by FOCUS independently of sorption properties of the compound. According to FOCUS, they have been calibrated against the results of tier III calculations. The key model for the estimation of run-off in FOCUS at tier III is Pesticide Root Zone Model (PRZM). Reichenberger et al. (2007) made a probabilistic analysis of losses caused by run-off and erosion using the PRZM and analysed losses dependent on sorption. For run-off,

the maximum losses were found for compounds with K_{OC} values in the range of 100–200 L/kg. For losses by soil erosion, the maximum numbers were found for compounds with maximum K_{OC} values. The results were evaluated by the German federal environmental protection agency and, meanwhile, were also implemented into their model EXPOSIT 3.0 used in German risk assessment for estimating pesticide losses caused by run-off (Umweltbundesam, 2011). Currently, this analysis is of use only in the central European zone, since only German environmental conditions were considered. However, it is recommended that the dependencies between important pesticide properties and run-off losses for all European zones be analysed in order to improve the information given by FOCUS (2001) (Table 28). As mitigation measures for run-off entries reaching surface waters, the EFSA PPR Panel (2013) recommends the use of vegetated buffer strips taken from FOCUS (2007). These mitigation measures for surface water are directly connected with deposition of residues to the respective terrestrial ecosystems (vegetated buffer strips). In Table 29 below, the reduction factors are re-calculated to give the reduction that is occurring at the respective distance only.

Table 28: Deposited fraction dependent on the position of the buffer strip

Buffer width (m)	Run-off fraction deposited (%)	Erosion fraction deposited (%)*
0–5	37	55
5–10	23	25
10–15	12	10
15–20	8	5

*: % refers to the reduction in the pesticides fraction sorbed to soil particles and leaving the field.

It is not possible to describe the deposition of pesticide in water by single exponential functions. However, the EFSA PPR Panel (2014a) gives exponential functions that could be used to derive deposited fractions for any distance for the water phase:

Table 30 shows examples for the acceptable distance in different seasons when 1 kg/ha was applied and the maximum acceptable load was calculated to be 0.01 kg/ha.

Substances with very high sorption constants (K_{OC} > 5,000 L/kg) are mainly transported via the sediment. For these substances, sorption via the particulate sediment phase (soil erosion) has to be considered with additional equations.

Table 29: Example of necessary run-off buffers (m) in different regions/seasons*

Region/Season	% of soil residue leaving the field**(a)	K_{OC}(L/kg)	Necessary distance(m)
North Europe, October–February	5	100	19.7
North Europe, March–May	2	100	7.3
North Europe, June–September	2	100	7.3
South Europe, October–February	4	100	16.5
South Europe, March–May	4	100	16.5
South Europe, June–September	3	100	12.3

*: Application dose 1 kg/ha, maximum acceptable load: 0.01 kg/ha, org carbon in soil 2%, concentration of suspended particles in run-off 0.01 kg/L.
**: Degradation in soil before run-off event not considered.
(a): % is the sum of losses due to run-off and soil erosion.

FOCUS (2007) recommended the reduction factors as being 90th percentile worst-case values for the reduction efficiencies of the buffer strips. Consequently, the related deposition fractions in the vegetated area should be considered as minimum deposited fractions. It is therefore recommended to re-evaluate the existing information on the efficiency of vegetated buffer strips with regard to worst-case situations for off-field areas taking into consideration the outcome of the workshop on Mitigating the risks of plant protection products in the environment (MAgPIE).

9. Effects Assessment

9.1. Introduction

As outlined in Section 6, SPGs have been developed for the following groups/species of in-soil organisms:

- earthworms
- enchytraeids
- microarthropods
- macroarthropods
- terrestrial gastropods
- nematodes
- Soil Bacteria and archaea
- mycorrhiza, other fungi and protozoa

This chapter describes the available study protocols for assessing the toxicity of PPPs to in-soil organisms. The aim is to evaluate whether the current methodology for effect assessment is appropriate to address the SPGs as reported above.

9.2. Choice of standard laboratory test methods for in-soil invertebrates

The objective of environmental risk assessment is to evaluate the likelihood that adverse effects may occur in the environment following exposure to a chemical. Standard laboratory tests aim at establishing the toxicity levels of a chemical on tested organisms. By characterising the toxicity, the way a chemical may interact with organisms and cause adverse effects should be taken into account. This means that, theoretically, all the potential routes of exposure (i.e. contact and oral) should be considered, depending on the physical and chemical properties of the substance and its mode of action. The test organisms can be considered 'models' and should be representative and sensitive enough for extrapolating the results to other species, when these results are used for risk assessment.

Many toxicity tests have been proposed and reviewed over the last decades (e.g. Lokke and Van Gestel (1998); Van Gestel (2012); and Pelosi et al. (2013)). Criteria for selecting an appropriate test for soil ecotoxicology have been proposed by Van Gestel (1997) and reported in Van Gestel (2012). Although these criteria were proposed for invertebrates, they concern general requirements that can be considered valid for microorganisms as well.

The criteria among others include:

1) Practicability, referring to the feasibility and cost-effectiveness of a test;
2) Acceptability, including aspects like standardisation, reproducibility and statistical validity of a test method as well as its broad chemical responsiveness;
3) Ecological meaning, including sensitivity and ecological realism of the test method.

The following, additional criteria need to be taken into account in order to obtain a balanced battery of tests (Van Gestel, 1997):

4) Representativeness of the ecosystem to protect, includes e.g. the representation of organisms having different life histories, representing different functional groups, different taxonomic groups and different routes of exposure;
5) Representativeness of responses, to make sure that responses measured really are relevant for the protection of populations and communities;
6) Uniformity, which refers to the possibility of applying all tests in a battery to the same test media.

The most relevant of these criteria for the purpose of this opinion are considered to be 1) the representativeness of the ecosystem to protect and 2) the representativeness of responses (points 4 and 5 above). The uniformity of the test systems with regard to test media and the possibility of scaling the toxicity endpoint to account for the bioavailability in different test media are discussed in Section 7.11.5.

In Section 6, SPGs have been developed using the overarching concept of Ecosystem Services, to identify key driver organisms that need to be protected in order to provide soil-ecosystem services that can be affected by the use of PPPs. Key drivers are therefore considered to be representative of

ecosystems to protect in terms of different functional and taxonomic groups and of different biological traits.

For invertebrates, a list of available laboratory-test methods was compiled according to the taxonomic groups of the key drivers (Appendix F). Appendix F includes formally standardised test protocols, but also protocols from open literature. It should be noted that the information presented has been compiled by the working group experts from public literature but has no claim of being exhaustive.

For microorganisms, functional endpoints are often used. Numerous methods are available for microorganisms; only the standard test (mentioned in the current data requirements) for nitrate formation is mentioned in Table 31, and other methods are referred to in Appendix G.

From Table 31, it can be noted that at least one laboratory test is available for key drivers in the main groups of invertebrates. The choice of a particular test species is discussed in Lokke and Van Gestel (1998). The authors showed that every test organism mentioned has an important role in the food web. Sensitivity of organisms to PPPs depends on the life stage, different life histories, etc. These aspects are discussed in Lokke and Van Gestel (1998) and in, e.g. Pelosi et al. (2014) for earthworms. It is also clear that choice of the test species and methods is a trade-off between the theoretically best species and practical aspects, like availability of an accepted guidance and handling in the laboratory.

Table 30: Overview of laboratory test methods for the identified key drivers (for soil ecosystem services potentially affected by pesticides). Bold indicates the key drivers as defined in Section 5. Standard laboratory tests according to EU Regulation 283/2013 and 284/2013 are made grey in the table. Test protocol: 1 = standardised test protocol available; 2 = test method available, not formally standardised

Taxonomic group			Ecosystem service						Habitat			Exposure					
	Key driver	Test organism/process	Biodiversity and geneticresources	Nutrient cycling	Pest control	Natural attenuation	Soil structure	Food web support	Soil	Litter	Test	Duration (d)	Soil contact	Soil pore water	Food	Litter	Endpoints/life stages
Prokaryotes	**Bacteria and Archaea**		X	X	X	X		X	X	X			X	X		X	
		N transformation	X	X					X		1	28–100	X	X			Nitrate formation
Eukaryotic micro organisms	**Mycorrhiza other fungi and protozoa**		X	X	X	X	X	X	X				X	X			
		Funneliformis mosseae (formerly *Glomus mosseae*)	X	X	X	X	X	X	X		1	14	X	X			Number of spores and germinated spores
Nematoda	**Nematodes**		X	X	X			X	X				X	X	X		
		Caenorhabditis elegans	X	X	X			X	X		1	1(4)					Mortality, growth and reproduction
Mollusca	**Terrestrial gastropods**		X	X			X	X	X	X			X	X	X	X	
Gastropoda		*Helix aspersa*	X	X			X	X		X	1	28	X	X	X		Survival, growth
Annelida Lumbricidae	**Earthworms**		X	X			X	X	X				X	X	X	X	
		Eisenia fetida fetida/andrei	X	X			X	X	X		1	56	X	X	X		Reproduction
Enchytraeidae	**Enchytraeids**		X	X			X	X									
		Enchytraeus albidus	X	X			X	X	X		1	42	X	X	X		Reproduction
		Cognettia sphagnetorum	X	X			X	X	X		2	70	X	X	X		Reproduction
Arthropoda	**Microarthropods**		X	X	X		X	X	X	X			X	X	X	X	

Taxonomic group		Test organism/process	Ecosystem service						Habitat		Test	Exposure					Endpoints/life stages
	Key driver		Biodiversity and geneticresources	Nutrient cycling	Pest control	Natural attenuation	Soil structure	Food web support	Soil	Litter		Duration (d)	Soil contact	Soil pore water	Food	Litter	
Collembola	Springtail	*Folsomia candida* and *F. fimetaria*	X	X	X		X	X	X	X	1	21–28	X		X		Survival, reproduction
		Isotoma viridis	X	X	X		X	X	X	X	2	56	X		X	X	Survival, growth
Arachnida: Laelapidae	Mites	*Hypoaspis aculeifer*	X	X	X		X	X	X	X	1	14	X		X		Survival, reproduction
		Platynothrus peltifer	X	X			X	X	X		2	70	X		X		Reproduction
		Oppia nitens	X	X			X	X	X		2	28	X		X		Reproduction
Arthropoda	**Macroarthropods**		X	X				X	X	X			X	X	X	X	
Chilopoda: Lithobiidae	Centipede	*Lithobius mutabilis*	X	X				X		X	2	28–84	X	X	X		Survival, sublethal effects
Diplopoda: Polydesmidae	Millepede	*Brachydesmus superus*	X	X				X		X	2	70	X	X			Survival, sublethal effects, reproduction
Crustacea: Isopoda	Woodlice	*Porcellio scaber*	X	X				X		X	2	4 survival growth), 10 (repr.)				X	Survival, growth, reproduction
		Porcellionides pruinosis	X	X				X	X		2	14	X	X	X		Survival, reproduction

Standard tier 1 testing according to current data requirements (Reg EU 283/2013 and 284/2013) includes earthworms (i.e. *Eisenia fetida/andrei*), microarthropods (i.e. the collembolan species *Folsomia candida*/*Folsomia fimetaria* and the mite *Hypoaspis aculeifer*) and soil microorganisms (i.e. N-transformation test). This means that mycorrhiza, nematodes, terrestrial gastropods, enchytraeids and macrodetritivores are not explicitly represented by the current standard test protocols. It is therefore discussed below for in-soil invertebrates and in Section 9.3 for soil microorganisms, whether present standard tests are representative for other key drivers.

As discussed above, the representativeness of the ecosystem to protect and the representativeness of responses are considered to be the key criteria for the selection of the standard test species/test system. The representativeness of the ecosystem to protect is evaluated based on the traits of the species/communities and the representativeness of responses based on available toxicity data. Considering the proposed SPGs, toxicity data on different species identified as key drivers have been compiled, when possible, trying to understand the representativeness of the current standard species and their use as model test species.

Representativeness of *Eisenia fetida*

Eisenia fetida is one of the current standard species for evaluating the toxicity of PPPs to in-soil organisms. It is evaluated below whether *E. fetida* can be considered a suitable model species, in terms of sensitivity, and can therefore represent in-soil non-arthropod invertebrates (i.e. other earthworms, enchytraeids, molluscs and nematodes) in the first-tier risk assessment.

E. fetida compared to other earthworms

E. fetida is an epigeic earthworm species, that lives in places rich in organic matter and it is mainly associated with compost and manure such as dung heaps. Endogeic earthworms like *Aporrectodea caliginosa* have different habitat, feeding strategy and life cycle. Differences in life cycle and habitat are two of the reasons why endogeic earthworm species are more difficult to maintain under laboratory conditions (Lokke and Van Gestel, 1998).

Regarding the suitability of *E. fetida* as a sensitive indicator species with regard to toxicity of PPPs on earthworms, Frampton et al. (2006) reported that *E. fetida* was mostly less sensitive than other Lumbricidae, based on an analysis of a rather limited data set (13 substances) of acute toxicity endpoints. Pelosi et al. (2013) suggested *A. caliginosa* as an indicator species after demonstrating a higher sensitivity of *A. caliginosa* and *Lumbricus terrestris* than *E. fetida* to PPPs. However, the analysis focused only on acute toxicity data since less reproductive studies were available to do such a meta-analysis (data not considered comparable, i.e. same earthworm development stage, active substance, type of substrate, pesticide addition, applied organic matter, and experimental duration). According to Daam et al. (2011), whose evaluation included both acute and chronic toxicity data from the US EPA ecotox database, the sensitivity of *E. fetida* appeared to be similar for insecticides, fungicides, and other compounds or slightly greater (for herbicides) compared with other Lumbricidae. The data presented in the paper of Daam et al. (2011) did not specifically allow comparing chronic sensitivity of different earthworm species.

Pelosi et al. (2014) reviewed public literature on acute and chronic effects of pesticides on earthworms including effects in the laboratory for authorised pesticides. The review done by Pelosi et al. (2014) allows a comparison of the chronic sensitivity of some earthworm species, since some studies were done under comparable lab conditions (listed in Table 32).

Table 31: Comparison of chronic sensitivity of different Earthworm species based on published literature for pesticides registered in Europe (adapted from Pelosi et al., 2014)

Reference	Species	Studied parameters	Active substance	Substrate	Organic material	Application method	Main Results
Bauer and Römbke (1997)	*Eisenia fetida, Aporrectodea longa, Lumbricus terrestris*	Mortality, biomass and reproduction	Amitrol/diuron	Natural and artificial	Cow manure and dried leaves	Sprayed and mixed	No evidence of any effects
Kreutzweiser et al. (2008)	*E. fetida, D. octaedra*	Survival, weight and cocoon production (and leaf consumption)	Imidacloprid	Litter	Litter and leaf material	Sprayed and mixed	*D. octaedra*, more sensitive than *E. fetida*. Significant weight losses among survivors of *D. octaedra* at 3 mg/kg. No effects on cocoon production among survivors at 3 mg/kg. *E. fetida*, significant weight losses at 14 mg/kg
Ma and Bodt (1993)	*A. caliginosa, A. longa, E. fetida, Eisenia veneta, L. terrestris, Lumbricus rubellus*	Mortality and reproduction	Chlorpyrifos	Natural and artificial	Sphagnum peat or leaves of alder	Mixed	Sensitivity Eisenia < Aporrectodea < Lumbricus. Reproduction more sensitive than survival. Substrate influence species sensitivity. Acute and chronic differences in sensitivity within one order of magnitude
Viswanathan (1997)	*E. andrei, L. terrestris*	Biomass, cocoon production, excretion	Terbuthylazine	Artificial	Alfalfa	Mixed	Reduction in juvenile number for *L. terrestris* at high doses. For *E. andrei*, reduction in cocoon production and biomass, increase in cocoon sterility with increasing duration and pesticide concentration. Comparison between the species in the presented experimental design is considered questionable since three generations were followed for *Eisenia* and only one generation for *L. terrestris*. Furthermore, no numbers of earthworms or cocoons are reported for *L. terrestris*, nor any experimental design is reported
Dittbrenner et al. (2012)	*E. fetida, A. caliginosa, L. terrestris*	Heat shock protein (HSP) 70 protein level and avoidance behaviour	Imidacloprid	Reference test soil		Mixed	HSP protein quantity is not a good biomarker of imidacloprid toxicity. *E. fetida* showed the strongest response of HSP protein levels. Significant avoidance behaviour but different between species

The few references allowing a direct comparison of chronic sensitivity of different earthworm species do not allow us to draw any robust conclusion about the sensitivity of *E. fetida* compared with other species but indicate that its sensitivity may also be lower for chronic effects. Pelosi et al. (2013) suggested that the more rapid excretion and higher metabolic rate of *E. fetida* could be the reason for a potentially lower sensitivity of *E. fetida* compared with other earthworm species, according to available literature.

In conclusion, data from the literature indicate that *Eisenia* species may not be the most sensitive species compared to other earthworms. Further research is, however, required in order to reach a robust conclusion on this issue, especially considering chronic sensitivity. For the time being, the PPR panel still recommends the widely used standardised test with the easily culturable model species *E. fetida* to be used in risk assessment considering an appropriate (calibrated) assessment factor. In parallel, it would be relevant to work at developing chronic laboratory test protocols to enable a robust conclusion on the chronic sensitivity of *E. fetida* with other species.

Eisenia fetida compared to Enchytraeids

Regarding the suitability of *E. fetida* as a sensitive indicator species also covering potential effects on enchytraeids, Jarratt and Thompson (2009) discuss that enchytraeids might be more suitable as standard test organisms than Lumbricidae, since tests on enchytraeids have practical advantages. Their comparison of acute and chronic toxicity of different chemicals to earthworms and enchythraeids, however, indicates that Lumbricidae were either similarly or more sensitive than enchytraeids overall, based on mortality and reproductive endpoints. In their comparison, there was also a small number of chemicals for which enchytraeids were > 5 times more sensitive than earthworms.

The Panel concludes that even though in some cases enchytraeids may be more sensitive it is likely that the response of *E. fetida* will be representative for enchytraeids and that remaining uncertainties can be addressed by an appropriate (calibrated) assessment factor. Therefore, it is proposed to use *E. fetida* as surrogate test species for enchytraeids taking into account an appropriate assessment factor.

Eisenia fetida compared with gastropods

Limited data are available for terrestrial gastropods, that might allow us to evaluate whether they can be considered covered by the current battery of toxicity tests for PPPs. Terrestrial gastropods are considered to be suitable bioindicators for metals (e.g. Cortet et al., 1999; Viard et al., 2004; De Vaufleury et al., 2006) and have been used frequently for *in situ* biomonitoring of sites contaminated by heavy metals (see e.g. Cortet et al., 1999). Various studies have been performed assessing the effects of different heavy metals on growth, food consumption, fecundity, mortality and molecular stress responses in terrestrial molluscs, mainly snails (Cortet et al., 1999; Triebskorn, 2009; De Vaufleury, 2015). For the retrospective risk assessment of metals, a standard test is requested to assess the soil quality using the vineyard snail *Helix aspersa* Müller (ISO, 2006). In the guideline, a test procedure to assess the effect of exposure to contaminated soil and a procedure to assess the effect of contaminated food mainly on growth (but also lethality) of juveniles of *H. aspersa* are described. This test is well qualified to assess the effect of metal contamination (Triebskorn, 2009) but as emphasised in the guideline itself, the test is not adequate to test volatile compounds. (Sverdrup et al., 2006) showed that it is not very suitable to assess the effects of PAHs.

There is less literature available on the effects of organic chemicals on terrestrial gastropods. Some studies investigate the side-effects of non-molluscicidal organic pesticides on snail and slug individuals; these studies consider contact exposure on contaminated surfaces, exposure via oral uptake of contaminated food or contact exposure in contaminated soil (e.g. according to ISO 15952) and record mortality, (shell) growth, sublethal signs of effect (e.g. body swelling, lethargy), histological changes or effects on enzyme activity (Rorke et al., 1974; Schuytema et al., 1994; Coeurdassier et al., 2001, 2002; Triebskorn et al., 2005; Druart et al., 2011; Hartnik et al., 2008).

There are also a few experiments available studying the effects of pesticides on terrestrial gastropod egg development (Iglesias et al., 2003; Druart et al., 2010, 2012). The test protocols allow studying effects on the embryonic development of land-snail eggs and the hatching success. Bioassays can be performed testing exposure via solid phase (contaminated soil) as well as via liquid/gaseous phase. The egg stage of terrestrial molluscs usually takes place in soil and therefore solid phase bioassays have a high ecological relevance for terrestrial gastropods.

Table 33 summarises studies from public literature testing the effects of pesticides on terrestrial gastropods exposed via spiked soil that allowed direct comparison with standard toxicity data. An overview of most available literature is provided by De Vaufleury (2015).

Table 32: Overview of tests with terrestrial gastropods compared with standard toxicity data

Reference	Test design	Results for tested organic pesticides	Comparable chronic regulatory standard toxicity data
Coeurdassier et al. (2002)	Juveniles of *H. aspersa* exposed via ISO substrate (spray application) to dimethoate for 7 days	Dimethoate (technical a.s.) EC_{50} growth = 150 mg/kg soil EC_{10} growth = 9.7 mg/kg soil	NOEC *E. fetida* = 2.87 mg a.s./kg soil dw
Druart et al. (2012)	Eggs of *H. aspersa* exposed via natural soil and ISO soil (2.9% and 10% OM content, respectively) to tebuconazole (formulation Corail) and glyphosate (formulation Bypass) for ca. 20 days	Tebuconazole (formulation Corail) tested: EC_{50} = 0.8(nat-soil)/7.8(OECD sub) mg a.i/kg soil NOEC = 0.9/5 mg a.i./kg soil Glyphosate (formulation Bypass) tested: EC_{50} = 219(nat-soil)/> 300(OECD sub) mg a.i/kg soil NOEC = 178/> 300 mg a.i./kg soil	Tebuconazole (formulation Folicur EW 250 – equivalent to Corail): NOEC *E. fetida* < 1.5 mg a.s./kg d.w. soil (10% OM, spray application) NOEC *Hypoaspis aculeifer* = 56.2 mg a.s./kg dw soil* No chronic studies testing the effects of the formulation Bypass (a.i. Glyphosate) towards in-soil organisms were available (please refer to EFSA conclusion Tebuconazole, EFSA Journal 2014;12(1): 3485 [98 pp.])
Hartnik et al. (2008)	Juveniles of *H. aspersa* exposed to Alpha-cypermethrin, technical via natural Norwegian soil according to ISO guideline. Effects towards *E. cypticus*, *E. fetida* and *F. candida* tested according to standard guidelines	Sensitivity *E. cypticus* > *E. fetida* > *F. candida* > *H. aspersa,* based on EC_{10} (growth for *H. aspersa* and reproduction for all other species) Sensitivity differed less than by a factor of 5	

EC_{10}: Concentration at which 10% effect was observed/calculated European and Mediterranean Plant Protection Organization; EC_{50}: Concentration at which 50% effect was observed/calculated European and Mediterranean Plant Protection Organization; NOEC: no observed effect concentration.

The amount of data available for comparing sensitivity of terrestrial gastropods and earthworms is too limited to draw a conclusion. In two of the three available studies for which a comparison of the sensitivity between gastropods and *E. fetida* could be made, *E. fetida* was more sensitive; in the third study, the gastropods were more sensitive. It should be noted that, for adult gastropods, exposure to pesticides via foliage and litter (oral and contact exposure) could present major pathways of exposure. There are several publications available testing also oral/contact exposure and the possibility to test oral exposure is also included in ISO 15952. However, since the exposure via foliage and litter (oral and contact exposure) is not covered by current standard testing requirements, it cannot be assumed that the toxicity towards terrestrial gastropods via these pathways is covered by current standard toxicity tests.

It is considered a critical issue that standardised test systems recommend using relatively large snail species, since the vast majority of land snail species are tiny (< 1 cm diameter in greatest dimension) and some even have maximum diameters of < 1 mm (see e.g. Sturm et al., 2006). Due to their smaller ratio of surface area to volume, these smaller species may be more susceptible to pesticide exposure than the current standard test species.

The Panel concludes that based on the limited amount of data available, no conclusion can be drawn about whether the standard test with *E. fetida* could be protective for gastropods, taking into account an appropriate (calibrated) assessment factor. It is also noted that potentially major exposure pathways, i.e. contact and oral exposure via foliage and litter, are not covered by standard testing requirements. At this stage, however, a suitable test system or a suitable testing strategy for gastropods cannot be recommended. For the test systems presented, it is as yet unclear whether the responses of the tested species/life stages are representative of the majority of terrestrial gastropods. Further research is required on this issue with the aim of developing suitable test systems to be used in regulatory practice.

E. fetida compared with nematodes

The bacteria-feeding nematode *Caenorhabditis elegans* Maupas, (Rhabditida: Rhabditidae) has almost ideal properties (e.g. easy to culture, simple body plan, and short generation and life cycle) to be used as a model organism in genetics, developmental biology and medical biochemistry. The effects of chemicals on *C. elegans* at various organisational levels (molecular, organs, whole organism, and population) have been described. Several types of toxicity assays are available, including whole-organism endpoints such as growth, reproduction and mortality, feeding inhibition, as well as molecular assays measuring the induction of stress-response genes. These assays also include a standardised chronic soil toxicity test (endpoints: growth and reproduction; 96 h; ISO 10872).

Apart from *C. elegans*, various other soil and marine nematode species have been utilised to assess the toxicity of xenobiotic compounds, such as pesticides. The nematode *Panagrellus redivivus* detects toxin concentrations that affect moulting and nematode size through stimulation, inhibition or lethality, enabling it to be used as a biomonitor (Neher, 2001). It has also been used to ascertain toxic effects of several hundred single chemicals (Neher, 2001). The most sensitive species are classified as cp-4 or cp-5 in the coloniser–persister classification of Bongers (1990).

There are very few publications comparing the sensitivity of nematodes versus current standard laboratory test organisms for pesticides. We found overall only few data that allow such a comparison of toxicity in the laboratory.

For the organic contaminants quinoline, acridine, phenazine, 1,10-phenanthroline and toxaphene both *E. fetida* and *F. candida* showed a similar or higher sensitivity than *C. elegans*, for chlorinated paraffin *F. candida* was more sensitive and *E. fetida* was less sensitive than *C. elegans* (Bezchlebová et al., 2007a,b; Sochova et al., 2007). These data are however only based on the parameter mortality and whereas the standard organisms *E. fetida* and *F. candida* were tested in OECD standard soil, *C. elegans* was tested in natural soil. Also the authors compared mortality at 28 days (*E. fetida* and *F. candida*) and mortality at 48 h (*C. elegans*). Considering much shorter duration of tests with *C. elegans*, it is however remarkable that the sensitivity of the species is sometimes in the same range than for *E. fetida* and *F. candida*,. In a study by Höss et al. (2009), *C. elegans* showed in several cases higher sensitivity to PAH contamination in soils than *E. fetida* and *F. candida* considering reproductive effects. This indicates that Nematodes may add non-redundant information to the current test battery.

Regarding the sensitivity of *C. elegans* towards other species, Boyd and Williams (2003) reported an average sensitivity of *C. elegans* compared to the two other Rhabditidae species *P. pacificus* and *P. redivivus* towards copper in soil considering mortality and reproduction. However, all three species are considered as cp1 species in the coloniser–persister classification of Bongers (1990) that are

considered the least susceptible nematode group to toxicants due to their traits. Zhao and Neher (2013) compared the sensitivity of *C. elegans* to *P. pacificus* towards the pesticide Acetochlor on agar medium and found a ca. fivefold higher acute sensitivity of *P. pacificus* and also a consistently higher sensitivity considering reproduction over three generations. Haegerbaeumer et al. (2016) studied the 48 h survival of *C. elegans* and other aquatic nematodes in microcosms following exposure towards zinc. Together with other cp-1 species *C. elegans* could be located in the lower third of the sensitivity distribution being less sensitive than the majority of species.

The most appropriate species would probably be predatory nematodes with K-strategies, e.g. *Mononchus* spp., but at the moment no standardised laboratory test is available. Predatory nematodes, such as Mononchidae, feed on other nematodes or on invertebrates in the soil. They are a very sensitive group of nematodes and are not able to adapt quickly to environmental disturbance. They were assigned a cp-4 value in the coloniser–persister classification by Bongers (1990) and to feeding group 5 by Yeates (2003). Nematodes such as Longidoridae, Discolaimidae and Thornenematidae that belong to cp-5 can also be potential bioindicators. These nematodes have the longest life spans of all the groups (Ferris and Bongers, 2009). Nematodes in the group also demonstrate the lowest fecundity, lowest metabolic rates and sluggish movement. They are particularly sensitive to toxins, as well as to other disturbances within their environments, due to their permeable cuticles (Ferris and Bongers, 2009).

Studies have focused on the effects of herbicides on plant-parasitic nematodes, especially root-knot nematodes and cyst nematodes (Browde et al., 1994; Dewar et al., 2000; Gilreath and Santos, 2004). In a recent review, Daam et al. (2011) found that nematodes are more sensitive to fungicides (copper sulfate and cupric chloride) than *E. fetida.* This comparison was based on toxicity values of 14 nematode taxa. However, when the effects of copper compounds were tested by using single nematode species, toxicity values were variable compared to those of *E. fetida*.

Nematodes are often chosen as bioindicators of the health status of all in-soil organisms. Zhao and Neher (2013) indicated that herbicides decreased abundance of both fungivores and predators; however, abundance of bacterivores, plant parasites and omnivores increased. Overall, total nematode abundance tended to increase in response to herbicide application.

Although there have been many studies about the interaction of agrochemicals and entomopathogenic nematodes (EPNs), there are limited toxicity data allowing a direct comparison of the sensitivity of EPNs with other model organisms (Koppenhofer et al., 2003). Several studies with insecticides (Garcia del Pino and Jove, 2005) and herbicides (Garcia del Pino and Morton, 2010) have reported absence of sublethal effects of agrochemicals on EPN biology.

In conclusion, as stated in Section 3, nematodes are an important and diverse group of in-soil organisms. Standard laboratory test methods are available and could provide a non-redundant addition to the current testing. Tests can be more quickly performed than for other in-soil invertebrates and could yield useful toxicity information. However, available data do not allow to conclude on an appropriate laboratory test species for nematodes and whether an addition of nematodes to the tier 1 test battery would be useful and which species can be tested to predict the response of other Nematode species. This requires further research.

Conclusion on the representativeness of *E. fetida* for non-arthropod soil invertebrates

The comparison of the sensitivity of *E. fetida* to other non-arthropod invertebrates key-drivers shows that limited data are available to compare directly the chronic toxicity of pesticides to different organisms. Available evidence indicates that the response of *E. fetida* might not always be representative nor cover the toxicity of pesticides to other key drivers. It should be noted that, for nematodes, a comparison of sensitivity with *E. fetida* was not possible. With respect to representativeness of *E. fetida* in terms of lifestyle, exposure routes and life cycle it should be noted that *E. fetida* is an epigeic species and does not reflect the traits of, e.g. endogeic species. However, currently available data, do not allow us to propose an alternative species as a sensitive indicator.

9.2.1. Representativeness of *Folsomia candida, Folsomia fimetaria* and *Hypoaspis aculeifer* for in-soil arthropod invertebrates

For in-soil arthropod invertebrates it was evaluated whether *F. candida/F. fimetaria* and *H. aculeifer* are suitable species to represent in-soil arthropod invertebrates (i.e. other springtails and mites other groups that are not tested, e.g. macroarthropods) in the first-tier risk assessment.

F. candida and *F. fimetaria* compared with other springtails

No systematic studies have been published on the sensitivity of *F. candida* and/or *F. fimetaria* compared with other collembolans. Table 34 shows three studies from the public literature. From these, with the exception of the experiments with picoxystrobin in Schnug et al. (2014), it seems that tests on *F. candida* or *F. fimetaria* with an appropriate assessment factor could be representative of other Collembola. According to the Schnug et al. (2014), the absence of effects on *F. fimetaria* might be due to food and habitat preferences, causing a much lower exposure. However, it is uncertain whether these effects would be also absent in a standard laboratory toxicity test.

Table 33: Overview of tests with springtails, comparing toxicity between species

Reference	Test design	Results for tested organic pesticides
Wiles and Frampton (1996)	Bioassays, field contaminated soil was studied in the lab. Test was conducted on four species (*Isotoma viridis*, *Isotomurus palustris*, *Folsomia candida* (Collembola: Isotomidae) and *Sminthurus viridis* (Collembola: Sminthuridae) and three substances: chlorpyrifos, cypermethrin and pirimicarb	Residues of cypermethrin and pirimicarb were of low toxicity, causing less than 10% mortality; residues of chlorpyrifos were toxic to all four species of Collembolan (from most to least susceptible) *S. viridis* > *F. candida* > *I. palustris* > *I. viridis*
Schnug et al. (2014)	Spiked soil, four species *Proisotoma minuta*, *Heteromuru nitidus*, *Folsomia fimetaria* and *Protaphorura fimata* tested with esfenvalerate, picoxystrobin and triclosan in a soil multispecies test system	For esfenvalerate differences in LC_{50} between species were within factor of 2. All species were not sensitive for triclosan, for picoxystrobin large differences in sensitivity (5 orders of magnitude between LC_{50} of most (P. firmata) and less sensitive species (*F. fimetaria*)). This difference might be due to food and habitat preferences
Lokke and Van Gestel (1998)	Standard test, three species *F. fimetaria*, *F. candida* and *Isotoma viridis* with copper chloride, LAS and dimethoate	Direct comparison was difficult because endpoints and soils are different between tested in-soil organisms. Generally, no systematic differences were found between the three species

In conclusion, very little data are available to compare the sensitivity of *F. candida* and *F. fimetaria* with other springtails. The few available data indicate that *F. candida* or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity and it is therefore recommended to use it as a surrogate test species for Collembola considering an appropriate assessment factor. In one case, *F. fimetaria* appeared to be insensitive compared with other species, but this might be a consequence of food and habitat preferences combined with the test conditions.

H. aculeifer compared with other mites

Lokke and Van Gestel (1998) compared the sensitivity of the herbivorous, oribatid mite *Platynothrus peltifer* with the predatory mite *H. aculeifer* for copper chloride, LAS and dimethoate. The results show that EC_{50} for reproduction does not differ by more than a factor of 2. For *Oppia nitens*, test methods were also available (Princz et al., 2010), however, no data were found that allow comparison of the sensitivity of this species with other mite species.

Since hardly any data were found comparing the sensitivity of *Hypoaspis aculeifer* with other soil mites, no conclusion can be drawn concerning the representativeness and further research is required on this issue.

F. candida compared with *Hypoaspis aculeifer*

From the EFSA conclusions on active substances, chronic toxicity data on *Folsomia candida* and *Hypoaspis aculeifer* were extracted. For completeness, sub-lethal toxicity data on *Eisenia* sp. were also extracted.

For 51 cases and 30 PPPs (10 herbicides, 11 fungicides, 8 insecticides, one acaricide), long-term toxicity data both on *Folsomia candida* and *Hypoaspis aculeifer* were available (see Figure 24). *Hypoaspis* was more sensitive than *Folsomia* for 8/51 compounds (15.7%). However, for three cases out of those eight, *Eisenia* was the most sensitive in-soil organism among the species tested. *Hypoaspis* showed a similar sensitivity than *Folsomia* for 22 compounds out of 51 (41%). These results

were confirmed by Owojori et al. (2014), who reported lower to intermediate sensitivity of *H. aculeifer* in comparison with other in-soil organisms for which standardised tests are available (*E. fetida*, *F. candida*, and *Enchytraeus albidus*). Huguier et al. (2015) also concluded that, compared with other in-soil meso-fauna invertebrates, mites were in general as sensitive or less sensitive than other test species, depending on the studied endpoints and chemicals. The status of *H. aculeifer* as the only predator among organisms for which a standardised protocol is available, however, highlights its usefulness in ecotoxicological laboratory studies. Please see Appendix H for more information about the data used in the analysis. In the standard laboratory test, however, *H. aculeifer* is not exposed via the route food uptake since it is fed with clean prey organisms. For exposure via soil, *H. aculeifer* does not always add information concerning the toxicity of in-soil organisms to tested chemicals compared with the test with *F. candida.* It is therefore recommended to study the possibility of adapting the test guideline in order to take the food-uptake route into account, which would be relevant for a predator such as *H. aculeifer*. Further research is needed in order to assess the sensitivity of *H. aculeifer* since it is not clear whether *H. aculeifer* is representative of other soil mites. Since *F. candida* is the most sensitive species in most of the cases, the mode of action of the substance does not seem to be determinant of the response when comparing *F. candida, H. aculeifer* and *E. fetida.*

In conclusion, it was found that *H. aculeifer* is the most sensitive test species for only 3/51 cases. This result indicates that it might be sufficient to test *E. fetida* and *F. candida* with the present test design. *H. aculeifer* represents another trophic level (predator species) in the standard test battery. Since it remains unclear if *H. aculeifer* itself is a good representative for soil mites and the feed-uptake route is not included, it is recommended to adapt the test protocol accordingly.

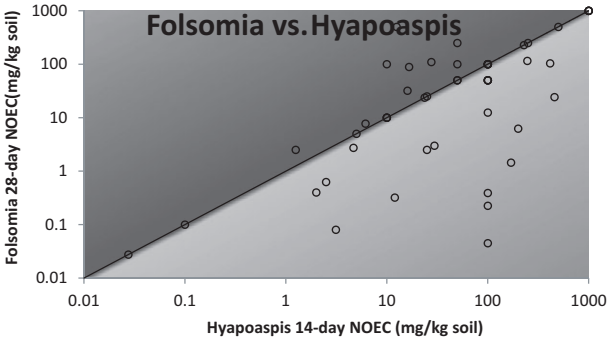

Figure 24: Comparison of sensitivity to a set of PPPs (active substances, metabolites or formulations) of *Folsomia candida* and *Hypoaspis aculeifer*. The solid line indicates a 1:1 relationship between *Folsomia candida* and *Hypoaspis aculeifer*. The light grey area in the figure indicates where *Folsomia* is more sensitive to pesticides

Folsomia candida compared with Isopods

For the comparison of the sensitivity of *F. candida* with Isopods, a review study was done based on literature (ISI WoK) and database search (US-EPA, PPDB database, PAN pesticide database).

Toxicity data on Isopods and Collembola were found for 13 compounds (see Table 35 and Table 36). Most of the toxicity data reported for isopods were based on mortality or parameters related to individual growth and feeding performance. Data reporting effects on reproduction and avoidance behaviour are scarce. The best represented isopod species is *Porcellionides pruinosus*, a species with a widespread distribution, being very abundant in southern European and tropical countries. This is considered an 'in-soil' species, since it can be found in the soil and not predominantly in litter, unlike most isopod species. It is also abundant near agricultural areas. For Collembola, toxicity data found were mostly on mortality and reproduction, with few references on avoidance behaviour. As expected, *Folsomia candida* was the best represented species.

The analysis of the toxicity data for Isopoda was done first by comparing the toxicity considering exposure via food and soil. Data for the different isopod species were pooled and differences between soil types were taken into account when possible. When more than one value exists for the same parameter and same exposure pathway, the geometric mean was calculated.

Table 34: Number of bibliographic records found per isopods and collembolan species and different PPPs

	Circoniscus ornatus	*Porcellio dilatatus*	*Porcellio scaber*	*Porcellionides pruinosus*	*Folsomia candida*	*Folsomia fimetaria*	*Orchesella cincta*	*Sinella curviseta*
Abamectin			5		4	2		
Atrazine				1	2		2	
Benomyl				8	10			
Carbofuran				3	4			
Copper				2	1	1		
Diazinon			14	6	4			
Dimethoate		1	8	6	14			
Endosulfan		1			3			
Glyphosate				1	3			
Imidacloprid			9		4			
L-cyhalothrin	5			18	3			1
Lindane				5	6			
Spirodiclofen				1	1			

Table 35: Acute and chronic endpoints measured in Isopoda and *Folsomia candida* for several PPPs

	Isopods (food)		Isopods (soil)		*Folsomia* spp.	
Abamectin			LC_{50} (Nat soil)	71	LC_{50} (Nat soil)	5.1
			NOEC_growth (Nat soil)	3	EC_{50} (Nat soil)	1.4
Atrazine			AC_{50}* (Nat soil)	153.1	NOEC_repro	40
					LOEC_repro	80
					EC_{50} (Nat soil)	43.2
Benomyl	LC_{50}	34,679.7	LC_{50} (Sandy soil)	1,068.8	NOEC_mort (Nat soil)	1
	NOEC_growth	1			LC_{50} (OECD)	13–18
					EC_{50} (OECD)	8–11
					EC_{50} (Nat soils)	2–10
					AC_{50} (OECD)	4–15
Carbofuran	LC_{50}	486	LC_{50} (Sandy soil)	31.02	NOEC_repro (OECD)	0.01
					LC_{50} (Nat soils)	0.06–0.09
					EC_{50} (Nat soils)	0.06–0.12
Copper			AC_{50} (Nat soil)	922.1	AC_{50} (OECD)	18
					EC_{50} (Nat soil)	1200
Diazinon	LC_{50}	182.6	LC_{50} (Sandy soil)	3.34	LC_{50} (OECD)	0.1
	NOEC_growth	> 100			NOEC_repro (OECD)	0.01
	NOEC_feeding	> 100			EC_{50} (Nat soil)	0.288
Dimethoate	LC_{50}	> 75	LC_{50} (Nat soil)	34	LC_{50} (Nat soil)	1.5
			LC_{50} (OECD)	44	LC_{50} (OECD)	0.6
			EC_{50}_growth (Nat soil)	17.5	NOEC_repro (Nat soil)	2.7
			EC_{50}_growth (OECD)	41.2	EC_{50}_repro (Nat soil)	0.77
			AC_{50} (Nat soil)	33.2	NOEC_repro (OECD)	2.7
Endosulfan	NOEC_feeding	50			LC_{50} (Nat soil)	0.08
					AC_{50} (Nat soil)	0.5
					EC_{50} (Nat soil)	0.05
Glyphosate			AC_{50} (Nat soil)	39.7	EC_{50}_repro (Nat soil)	0.42
					NOEC_avoid (Nat soil)	1.2
Imidacloprid	NOEC_mortality	> 25			LC_{50} (TAS)	20.96
	NOEC _growth	> 25			NOEC_mort (TAS)	10
	NOEC _feeding	10			EC_{50} (TAS)	0.06
					NOEC (TAS)	0.01
L-cyhalothrin			NOEC_mort (TAS**&OECD)	0.16	NOEC_repro (OECD)	7.6
			NOEC_mort (Nat soils)	0.17	EC_{50} (OECD 5%)	5.64
			LC_{50} (TAS&OECD)	0.61		
			LC_{50} (Nat soils)	0.31		
			NOEC_repro (OECD)	0.1		
			NOEC_repro (Nat soil)	0.1		
			EC_{50}_repro (OECD)	0.4		
			EC_{50}_repro (Nat soil)	0.13		
Lindane			LC_{50} (OECD)	80	LC_{50}	1
			AC_{50} (Nat soil)	35.3	EC_{50} (OECD)	0.13
					NOEC (OECD)	0.03
					EC_{50} (Nat soil)	0.8
Spirodiclofen			AC_{50} (Nat soil)	0.9	EC_{50}_repro (Nat soil)	0.65

LC_{50}: lethal concentration, median; NOEC: no observed effect concentration.
*: AC_{50}: concentration inducing avoidance in 50% of the tested animals
**: TAS: Tropical artificial soil.

For the four compounds where comparable data are available (mortality data only), contact exposure to soil was revealed to lead to more adverse effects for isopods than food exposure. Although data are scarce, toxicokinetic studies performed by Sousa et al. (2000) with lindane revealed that isopods were able to accumulate more when exposed via soil than when exposed via food (the reason was mainly the high excretion rates observed and the slower degradation of the compound in the soil matrix). Despite this trend, this comparison between exposure routes should be interpreted carefully since none of these studies considered another important exposure route for these animals, contact in litter.

The sensitivity of Isopoda and Collembola was compared for soil exposure only using the same parameters and measurement endpoints, directly for eight compounds (mainly acute data) and indirectly for five of the compounds. In this last comparison, the parameters assessed were not the same, but it is possible to infer some trend in sensitivity by looking at the differences between values obtained.

For seven out of eight compounds (abamectin, benomyl, carbofuran, copper, diazinon, dimethoate and lindane), Collembola showed a higher acute and chronic sensitivity than Isopodsa (several orders of magnitude higher). Indirect comparisons (looking also at chronic parameters) revealed a similar trend. The exception was L-cyhalothrin, where *P. pruinosus* was more sensitive than *F. candida* with effects on reproduction occurring at lower concentrations.

As indicated in Sections 5 and 6.2.4, isopods are key drivers for several ecosystem processes, especially those linked to organic matter decomposition and nutrient cycling. Although they can take up chemicals by being in contact with contaminated soil (via the cuticle), the main route of exposure for most isopod species is in litter. Isopods can take up chemicals via contact with moist litter surfaces, but their exposure is higher when they feed on contaminated litter material (Peijnenburg et al., 2012).

When considering effects of PPPs on isopods exposed via soil, existing data show that their sensitivity is covered by other test species, e.g. the collembolan *Folsomia candida* (see above). However, the assessment of effects via exposure to contaminated litter, especially via food consumption, should be considered when designing a more appropriate hazard assessment of these compounds.

Despite the absence of a fully standardised ecotoxicity test (i.e. ISO or OECD guideline) on isopods, there is a vast experience in the literature on using them as model organisms for ecotoxicological evaluations of PPPs, ranging from assessing individual and population-level parameters (e.g. Jänsch et al., 2005; Morgado et al., 2016; Vink et al., 1995; Zidar et al., 2012) to ecotoxicogenomic studies (e.g. Costa et al., 2013a,b). Most published studies with PPPs focus on three species (*Porcellio scaber, Porcellio dilatatus* and *Porcellionides pruinosus*) and address lethal effects and effects linked to food consumption (measuring consumption, assimilation and assimilation efficiency) and their direct effects on energy allocation (measuring energy reserves) and, ultimately on growth (measuring biomass changes) at an individual level (Drobne et al., 2008; Ferreira et al., 2015; Ribeiro et al., 2001; Stanek et al., 2006; Zidar et al., 2012). Some studies also address behavioural parameters (Engenheiro et al., 2005; Loureiro et al., 2005, 2009; Santos et al., 2010). Although all these parameters can influence the onset of reproduction, number of offspring and, ultimately, population growth, it would be important to have more information on the direct effects of chemicals on reproductive parameters. In fact, the number of studies addressing direct effects on isopod reproduction is quite rare (e.g. Jänsch et al., 2005).

All these aspects, allied to the need to assess effects to key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris, prompt the need to develop further a standardised test addressing both feeding and reproduction parameters. Despite the few papers on the optimisation of culture and test conditions (e.g. Caseiro et al., 2000), further research on the optimal isopod species, test design (e.g. test media, test duration, type of parameters), and litter material to use is needed. The extensive information already existing in literature could be a good starting point for a proposal for an ISO or OECD guideline needed to cover this group of key drivers via this particular and relevant exposure route to PPPs.

In conclusion, based on the available literature, *F. candida* may be protective for chronic effects on isopods when exposed via soil in the laboratory, taking into account an appropriate assessment factor. However, Isopoda are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is litter on the soil surface. Therefore, the panel recommends development of a standardised test addressing both feeding and reproduction parameters, in order to assess effects to key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

Conclusion on the representativeness of *Folsomia candida*/*Folsomia fimetaria* and *Hypoaspis aculeifer* for arthropod soil invertebrates

The few available data indicate that *F. candida* and/or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity. Since hardly any data were found comparing the sensitivity of *H. aculeifer* with other soil mites, no conclusion can be drawn concerning its representativeness for other mite species. Even though with regard to the representativeness for the ecosystem, *H. aculeifer* seems important since it is the only predator in the test battery for which a standardised test protocol is available, an analysis of its toxicological sensitivity indicated that it showed a relatively low sensitivity compared with *F. candida*. Based on the available literature, *F. candida* may be protective for chronic effects on isopods when exposed via soil in the laboratory.

However, none of these studies considered an important exposure route for these animals, i.e. contact in the litter on the soil. Therefore, no conclusion can be drawn on that respect. In order to take this important exposure route into account, the Panel recommends development of a standardised test addressing both feeding and reproduction parameters, to assess effects to key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris.

9.2.2. Conclusion and recommendations for the choice of the standard laboratory test system for invertebrates

A lot of data is available concerning the toxicity of PPPs to individual species of soil invertebrate. Only limited data are available, however, that allows a sound scientific comparison, due to differences in substance, test conditions, duration, exposure route and/or endpoint.

Making an overall comparison of the sensitivity of current standard test species, Frampton et al. (2006) and Daam et al. (2011) show that *E. fetida* is often not the most sensitive species when compared with soil arthropods, such as springtails, arachnids and isopods. Daam et al. (2011), compared the sensitivity of *E. fetida* in the standard laboratory test to the sensitivity of other standard laboratory test species. The authors concluded that testing only *E. fetida* was not predictive for a number of other species, and that the inclusion of *F. candida* in the test battery significantly lowered uncertainty due to interspecies variability, which has been implemented in the most recent version of the data requirements (Reg 283/2013 and 284/2013). No conclusion could be drawn, however, on the representativeness of the current test battery for many of the other relevant invertebrate key drivers also due to limited availability of toxicity data.

With respect to representativeness of *E. fetida,* in terms of lifestyle, exposure routes and life cycle, it should be noted that *E. fetida* is an epigeic species and does not reflect the traits of, e.g. endogeic species. A robust comparison of sensitivity between *E. fetida* and other earthworm species could rarely be performed. No comparison with *E. fetida* was possible for nematodes. Since currently available data do not allow us to propose alternative species, it is concluded, that for the time being *E. fetida* should be used as a representative species for non-arthropod invertebrates in first-tier risk assessment with an appropriate assessment factor. Research is required with the aim of developing suitable tier 1 test systems to complement current testing of non-arthropod soil invertebrates with regard to the sensitivity of test species and tested exposure routes.

The few data found, indicate that *F. candida* and/or *F. fimetaria* could be representative of other springtails with regard to their toxicological sensitivity. For the time being, *F. candida* and *F. fimetaria* are therefore considered suitable model species for springtails.

Since hardly any data were found comparing the sensitivity of *Hypoaspis aculeifer* with other soil mites, no conclusion can be drawn concerning their representativeness for mites, and further research is required on this issue. Even though with regard to the representativeness for the ecosystem, *H. aculeifer* seems important since it is the only predator in the test battery for which a standardised test protocol is available, an analysis of its toxicological sensitivity indicated that due to their comparably low sensitivity it might be omitted from the tier 1 test battery. However, the present protocol does not include an important exposure route for *H. aculeifer* contact via food. Therefore, it is recommended to adapt the first tier test with *H. aculeifer* to take food uptake into account, and to keep it as a first tier standard test in the adapted form.

Based on the available literature *F. candida* may be protective for chronic effects on isopods when exposed via soil in the laboratory and may be an appropriate representative species for isopods considering an appropriate safety factor. However, none of the present test methods considered an important exposure route for these animals, i.e. for isopods contact in the litter layer.

Isopods are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is the litter layer. Therefore, the Panel recommends to develop a standardised test addressing both feeding and reproduction parameters, to assess effects to key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

Overall, considering the available data, the Panel consider that the current test battery with the use of an appropriate (calibrated) assessment factor might cover the intra- and interspecies variability in soil, with the exception of the toxicity of in-soil organisms when exposed via food and via the litter layer as well as in the case of highly specific compounds. In those cases that pesticides may specifically act on certain groups, which do not have close relatives in the suggested standard test battery (especially in the case of nematodes and gastropods), it may not be possible to define an appropriate (calibrated) assessment factor since effects simply may not manifest in the standard systems. Therefore, the Panel recommends further research aiming at investigating the toxicity of PPPs on in-soil organisms other than the standard ones (*E. fetida*, *F. candida* and *H. aculeifer*) to allow a better estimation of their representativeness in terms of sensitivity compared to the other key drivers as identified in Section 5 (i.e. enchytraeids, isopods, nematodes, terrestrial gastropods). In addition, further research is needed on the sensitivity of in-soil organisms when exposed via food uptake and via the litter layer. It is recommended to adapt the test with *H. aculeifer* to take food uptake into account, to develop a standardised test with isopods, to take exposure via the litter into account. It is also important to find/develop suitable and representative test systems with nematodes and gastropods for tier 1 testing, which may be necessary for very specific compounds and add non-redundant toxicity information to the current test battery.

9.3. Choice of standard laboratory test methods for microorganisms

9.3.1. General considerations for microbes

A description of the available test methods for microorganisms is reported in Appendix I. Available ISO standards with potential relevance for soil microbes are listed in Appendix G.

While general toxicity-test systems using single microbial species or strains (e.g. Johnson et al., 2009; Dijksterhuis et al., 2011) can give information both on direct effects on the test organism and on specific toxicity mechanisms or degradation pathways, they are of limited use in prospective risk assessment for microbes in general. Their main disadvantage is the quite low representativeness of one species to the vast microbial genotypic and phenotypic diversity of soils. Also, a big majority of microorganisms currently cannot be cultured as pure isolates, but can only be studied in the context of more or less complex natural communities (e.g. Wagner, 2004). Hence, using field samples in mesocosm or microcosm experiments with entire communities has substantially higher environmental relevance and has recently been advocated (Puglisi, 2012); (ECHA, 2014; Karpouzas et al., 2014).

In community-level tests using (semi)field studies with natural soil, longer term chronic effects induced by pesticides can be determined, taking taxonomic and functional shifts in microbial communities into account. Endpoints can relate to function (activities or processes), biomass (total biomass, or biomass for taxonomic or functional sub-groups) or structural properties (community structure or diversity) (see Appendix I).

The big progress in development and adaption of molecular methods has lately facilitated descriptions of different aspects of *in situ* microbial communities, particularly concerning their structural properties. One established method is PLFA (phospholipid fatty acid analysis), where the PLFA composition of a sample gives a picture of the microbial community structure. Nucleic acid-based methods have bigger potential to give detailed information on the structure and diversity of microbial communities and the presence and abundance of specific phylogenetic or functional groups of organisms. In recent years, metagenomic approaches and 454 sequencing have been broadly introduced to studies of dynamics in soil microbial community structure (e.g. Feld et al., 2015). Besides giving information on the overall community structure or phylogenetic diversity, molecular methods can also provide data related to functional properties. For instance, quantitative PCR-based methods can be used to determine the abundance of particular functional genes (i.e. independently of the phylogenetic affiliation of the organisms carrying the genes), and hence subgroups of the microbial community potentially able to perform a specific process (e.g. Fang et al., 2014; Anzuay et al., 2015). Additionally, functional gene arrays (e.g. geochip based analyses) can give information on the

presence/absence of a large number of functional genes, and thereby give a fingerprint of the metabolic potential of microbial communities (He et al., 2012).

There is no doubt that modern molecular methods have a huge potential to yield valuable information on effects of environmental changes and stressors on microbial communities. However, performing the analyses and interpreting the large data sets often produced is scientifically complex and so far no such methods have been evaluated for use in a regulatory context.

Chemical pesticides (and other organic pollutants) can influence microorganisms in two opposite ways: (i) the compound is toxic to the microbe; and (ii) for a microbe exhibiting tolerance to the compound, it may act as a nutrient and energy source and, hence, stimulate growth of those organisms. A consequence of the possible dual effects of pesticides on different metabolic and functional groups of microbes is that spiking soil with pesticides commonly results in increases in microbiological parameters. Accordingly, in the terrestrial data set of the systematic literature review of responses of microorganisms to pesticides (Puglisi, 2012; external procurement funded by EFSA), a substantial part of the entries in the database represented significant increases. The summary evaluation of the data was reported separately for type of substance (fungicides, herbicides and insecticides) and type of assay (based on activity, biomass or community structure). Regarding effects on microbial activities, 39–47% of entries showed no significant effects, 21–32% significant decrease and 11–22% significant increase. For assays based on biomass, corresponding numbers were 26–55% (no effect), 11–23% (decrease) and 14–44% (increase). In the material analysed by Puglisi (2012), increases in response were more common for insecticides than for either fungicides or herbicides, both for the activity- and biomass-based endpoints. In the analysis of effects on endpoints based on community structure (using PCR-DGGE, PLFA, BIOLOG®, etc.), a different and qualitative approach was taken using three classes: significant changes, transient significant changes and no significant changes. The majority (80–95%) of the entries showed significant changes in community structure, while the remainder showed either no or transient effects.

It is important to keep in mind that, in community-based tests, a no-effect outcome in a functional endpoint after pesticide spiking to soil is a net response, and not proof that no populations have been affected in either a negative or a positive way. In community-based measurements of chronic effects, functional redundancy – i.e. if organisms performing a certain function are inhibited or eliminated, other organisms take over and fill the niche – can also hide effects on specific populations within functional groups, thereby complicating the interpretation of test outcomes.

9.3.2. Prospects for improving test strategies for microorganisms

Phylogenetic or functional groups as well as individual species of microorganisms contribute to several of the different ESs. To begin with, all soil microorganisms principally contribute to the ESs genetic diversity, biodiversity, cultural services and food-web support. Apart from this, many microbial groups also contribute to several other ESs. Taking the functional (and polyphyletic) group denitrifying bacterial taxa as an example, these bacteria will often also contribute to the ESs nutrient cycling, natural attenuation and possibly pest and disease control. Additionally, although knowledge regarding both the structural and functional properties of soil microbes is growing rapidly, for many functions and activities performed by soil microbes (especially common functions performed by many taxonomical groups), confining the functions to certain phylogenetic units is still a challenge.

Based on the above it is concluded that, for selection of the most relevant community-based microbial test systems, the best strategy seems to be to consider assays that cover as wide a range as possible of processes and key drivers, while it is hardly realistic to design a set-up containing specific assays for specific key drivers. The currently required community-based functional tests of effects on N transformation (the EU and North America) and on general microbial activity (CO_2 evolution; North America) take the activity of a wide selection of microbes into account, but are considered comparatively coarse and insensitive. MicroResp® is a recent development of the BIOLOG® method that has potential to give more detailed information regarding degradation capacity of a soil, and the effect of pollutants on degradation (Campbell et al., 2007). In contrast to the N-transformation and respiration tests, MicroResp® yields data on the degradation capacity in the sample of a number of different types of substrates. It describes the functional/physiological capacity for degradation and measures microbial activity as CO_2 evolution directly from, e.g., soil separately for each included substrate. So far, MicroResp® has not been used to study effects of pesticides on soil microbial processes but it is recommended that its capacity in that respect is determined in future research.

The aim of finding broad microbial tests that cover as much as possible of phylogenetic and functional variation may need to be balanced against the aim of sensitivity. For illustration, functional redundancy can be expected to contribute less to insensitivity when studying more specialised functions. One example is that potential nitrification has been found to be relatively sensitive to pollutants (Pell et al., 1998), since the ammonia-oxidising bacteria mediating the first step represent a small group of fastidious, slow-growing lithotrophic bacteria. In recent years, however, it has become evident that some archaeans can also oxidise ammonia in soil (Leininger et al., 2006). Redundancy is more likely for functions that are widely distributed among many microbial groups, meaning that if inhibitory effects are seen for such functions, it is likely that many different microorganisms have been affected.

Currently, there is no data requirement related to mycorrhizal fungi. Their importance in providing several important ecosystem services is, however, well known and reported in the literature. In addition, mycorrhizae provide specific functions (e.g. soil formation) that represent an exclusive trait of mycorrhizae in general and arbuscular mycorrhizal fungi in particular.

A standardised test with arbuscular mycorrhizal fungi was proposed by ISO (ISO/TS 10832:2009) where the acute effect of chemicals (or contaminated matrices) on spore germination of the arbuscular mycorrhizal fungal species *Funneliformis mosseae* (formerly *Glomus mosseae*) (strain BEG12) is assessed. The test is conducted over 14 days at 24°C on sand or OECD artificial soil, with the percentage of germinated spores, in relation to the number of recovered spores, being the measured parameter.

The test species (*F. mosseae* formerly *G. mosseae*) has been proposed as a model species to assess the toxicity of different chemicals due to its widespread distribution around the world and its high abundance to crop systems (Smith and Read, 2008). When testing different PPPs, however, Giovannetti et al. (2006) found that spore germination was only a sensitive parameter towards some of the fungicides tested but not to the tested herbicides. On the other hand, mycelium growth (also at the asymbiotic phase) proved to be a much more sensitive parameter, being inhibited by all fungicides and herbicides tested.

These results could indicate that, when using arbuscular mycorrhizal fungi as a test species, parameters other than spore germination could be assessed, increasing the informative value of the assessment. This could indicate some improvements in the existing protocol to accommodate the measurement of extra parameters. In a recent study, Mallmann (2016) demonstrated the practicability and reproducibility of the ISO protocol, together with its ability to be used with other arbuscular mycorrhizal fungi species (five species were tested) and to measure other parameters, including the growth of the asymbiotic mycelium. Moreover, since the sensitivity to some chemicals can vary between strains of the same species, studies like this one that focus also on evaluating inter- and intraspecies variation in sensitivity should be performed and the more appropriate test species and strain should be selected.

Conclusion

From the above and from Appendix I it can be concluded that a number of methods exist to measure soil microbial properties of soil in terms of abundance, activities/functions as well as community structure. A number of interpretation problems are identified, e.g. effects on functional endpoints might be diluted by functional redundancy; regarding structural endpoints, the interpretation in the sense of their connection with certain functions is hard to define.

Novel methods are continuously developed that are able to record a broad spectrum of functional and structural endpoints. These methods need further adjustment and standardisation for use in risk assessment of pesticides, but may become useful tools in the future.

Since the present test aimed at N transformation covers a number of processes, it is considered a relevant indicator, most obviously for the functions nutrient cycling and food-web support. In the present test design for agrochemicals, it is prescribed to test two dosages and to determine at different points in time whether effects are > 25%. When after 28 days effects are > 25%, the test can be prolonged to 100 days. For non-agrochemical substances, however, a dose–response design is prescribed. In order to assess whether the effects fit with the magnitude and the temporal scale mentioned in the SPG chapter, it is recommended to use a dose-effect design for agro-chemicals as well.

Activities of protozoa and many fungi are, to a substantial extent, covered by the N-transformation test, whereas, mycorrhizae are not incorporated. It is therefore recommended to add a test with mycorrhizal fungi. The ISO test with *Funneliformis mosseae* (formerly *Glomus mosseae*) allows for

adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design.

9.4. Conclusions and recommendations concerning the choice of lower tier standard test species

In summary, based on the analyses presented above it is recommended to use as standard test species in the first tier: *E. fetida*, *F. candida* and/or *F. fimetaria*, *H. aculeifer* and N-transformation. From Section 9.3 above it is clear that *E. fetida* and *H. aculeifer* might not be the most sensitive species. The available data did not allow drawing any conclusion on the sensitivity of those species when compared to other in-soil organisms. In some cases, adaptation of the current test system is proposed. Furthermore, it will be essential to use a calibrated assessment factor in the guidance document.

Exposure via food uptake is only partly included in the standard laboratory tests, since normally uncontaminated food is often provided after exposure. When food is an important exposure route, the test protocols might be adapted better to include this route. Therefore, it is recommended to adapt the test with *H. aculeifer* in order to take exposure via food into account. This can be accomplished by giving live food (e.g. young specimens of *Folsomia candida*) that has been feeding on yeast spiked with the PPP to be tested. Research is needed to infer properly the spiking doses and the exposure time of *F. candida* to the contaminated yeast, taking into account the toxicokinetics of the PPP in the collembola, in order to obtain the desired body residues in the food to be offered to the mites. It is also important to find/develop suitable and representative test systems with nematodes and gastropods for tier 1 testing, which may be necessary for very specific compounds and add non-redundant toxicity information to the current test battery.

Isopods are key drivers for several ecosystem processes and the preferential route of exposure for most isopod species is the litter layer or litter debris on the soil surface. Therefore, the panel recommends development of a standardised test addressing both feeding and reproduction parameters, in order to assess effects on key in-soil organisms having a relevant exposure to PPPs via consumption of litter debris. The available information on isopods renders this a good candidate for developing an ISO or an OECD guideline.

The present standard test species do not cover mycorrhizal fungi. Mycorrhizal fungi are considered very important for many ecosystem services. Due to their peculiarity, they are not considered covered by the N-transformation test. It is therefore recommended to add an additional standard test with mycorrhizal fungi. The ISO test with *Funneliformis mosseae* (formerly *Glomus mosseae*) allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design.

Most tests are designed for the most common modes of action. In general, for specific or new modes of action, it should be carefully checked whether the current standard test methods are sufficiently protective.

Most laboratory test methods are designed for spray applications, but can be adapted in order to test effects of soil fumigants, treated seeds and granules. For soil fumigants, experience is available for nematodes (see e.g. Ma et al., 2001, Zasada et al., 2007). For the off-field, an option for granules would be simulating exposure with dust particles.

9.5. Additional laboratory testing

When assessing active substances and PPPs, additional laboratory-toxicity data exceeding the regulatory requirements (Commission Regulation (EU) No 283/2013 and No 284/2013) might be available. Those may be due both to the legal obligations to submit scientific peer-reviewed open literature data and/or to the choice of the applicant to submit additional laboratory tests as possible refinements. This open literature data may be used in identifying additional toxicity data for relevant species, which are not captured in the standard test package. Additional data on relevant species can be used to address intra- and interspecies variability. From the analyses of the sensitivity of test organisms (see Section 9.2), used in the standard laboratory tests, it is clear that species may vary markedly in their sensitivity to PPPs and this difference may have a large contribution to the uncertainty of the assessment based on current standard test species.

This variation in direct toxicity can be described by constructing an SSD. The SSD is a statistical distribution estimated from a sample of laboratory-toxicity data and visualised as a cumulative distribution function. SSDs are used to calculate the concentration at which a specified proportion of

species is expected to suffer direct toxic effects. These concentrations, the hazardous concentrations, are expressed as HC_x values and represent the value that affects a specific proportion (x%) of species. For regulatory purposes, usually the median HC_5 is used, the hazardous concentration to 5% of the species tested. When compared with the first-tier effect assessment on the basis of standard test species, SSDs have the advantage of making more use of the available laboratory-toxicity data for a larger array of species. They describe the range of sensitivity rather than focusing on a single value, they enable estimates to be made of the proportion of the species affected at different concentrations, and they can be shown together with confidence limits showing the sampling uncertainty due to the limited number of tested species (EFSA PPR Panel, 2013). The concept of SSDs is applied in, e.g. aquatic risk assessment or terrestrial non-target-plant risk assessment. The SSD conceptual model was also discussed and considered as a useful tool by the ECHA/EFSA Topic Workshop (ECHA, 2015). However, at that workshop it was recommended that more guidance is needed on the applicability of SSD for in-soil organisms. In the guidance document for aquatic risk assessment (EFSA PPR Panel, 2013), other options are given as well, such as the geometric mean. The latter means that, when not enough data are available to apply a reliable SSD, the geomean of comparable toxicity data for species of the same taxonomic group could be used for further risk assessment (instead of the lowest, most sensitive endpoint). Further research is needed in order to draw conclusions about the use of this type of alternative for the in-soil risk assessment.

Frampton et al. (2006) described the sensitivity range of in-soil organism by using the SSD approach for acute toxicity data. It is clear from Table 31 and Appendix F that test methods addressing chronic toxicity are available for a number of additional species. It is, however, also clear that the number of test protocols is limited, especially within certain groups.

For additional information on the minimum number of toxicity data and other practical points (e.g. use of unbound values, etc.) for constructing a SSD, please refer to the EFSA PPR Panel (2013).

SSD might be a powerful tool to reduce uncertainty in the risk assessment linked to intra- and interspecies variability in sensitivity to PPPs. Contrary to aquatic organisms, however, there is only limited experience in combining toxicity data of in-soil organisms in SSD, especially for PPPs. Further research is needed with regard, in particular to combining toxicity data from different groups of in-soil organisms. According to the EFSA PPR Panel Aquatic Guidance (EFSA PPR Panel, 2013), the toxic mode of action of a PPP should be taken into account when constructing SSDs. Chronic toxicity data on different groups of in-soil organisms are, however, rarely available.

9.6. Additional test methods addressing specific questions and issues

9.6.1. Addressing transgenerational effects

Campiche et al. (2007) proposed a multigeneration test with *F. candida* to complement the risk assessment of insect growth regulators. Their results indicated that some endocrine disruptors had effects on several generations even though only the F_0 generation was exposed. A comparable test has been developed for the enchytraeid species *E. crypticus* (Bicho et al., 2015). The potential of trangenerational effects may present an uncertainty that could be addressed by additional testing. It should be investigated during the development of the guidance document how relevant transgenerational effects are for the response of field communities and how transgenerational effects differ between species. Ernst et al. (2016) proposed a multigeneration test with Collembola to address recovery in a laboratory study. This proposal is discussed in Appendix J.

9.6.2. Bioaccumulation

The assessment of potential effects of bioaccumulation or biomagnification on in-soil organisms is not included in the Regulation. The Regulation states that:

> 'An active substance, safener or synergist fulfils the bioaccumulation criterion where there is: evidence that its bioconcentration factor or bioaccumulation factor in aquatic species is greater than 5,000 or, in the absence of such data, that the partition coefficient *n*-octanol/water (log K_{ow}) is greater than 5, or evidence that the active substance, safener or synergist present other reasons for concern, such as high bioaccumulation in other non-target species, high toxicity or ecotoxicity'.

The trigger for the bioaccumulation criterion, however, is based on aquatic organisms only. Bioaccumulation in soil organisms (worms) is taken into account in order to assess the potential for secondary poisoning in birds and mammals (EFSA, 2009a).

In general, bioaccumulation often correlates with lipophilicity; thus, for organic chemicals, a log $K_{ow} \geq 3$ indicates a potential for bioaccumulation. For birds and mammals, this triggers the risk for secondary poisoning assessment for birds and mammals. In the aquatic environment, the bioaccumlative potential of a substance triggers a full life cycle study with fish (EFSA PPR Panel, 2013). According to the actual data requirements (EU VO 283/2013, Point 8.2.2.3), a log $K_{ow} > 3$ triggers a bioaccumulation study on fishes (BCF-study) and the outcome of such study is used for classification of the active substance as (non-/)bioaccumulative in the context of PBT-criteria. There is, at the moment, no corresponding study requested for soil organisms.

The guideline for bioaccumulation in terrestrial oligochaetes (OECD, 2010) is aimed at calculating the bioaccumulation factor (BAF) in order to assess this potential. Experimental data (Bruns et al., 2002) show a higher potential for bioaccumulation for enchytraeids than earthworms. The literature concerning bioaccumulation in in-soil organisms is aimed at concentrations that might affect predators, not at effects on in-soil organisms themselves (e.g. Gobas et al. (2015)). Effects of bioaccumulation are partly inherently included in the tests, since the test substance can accumulate in the test organism during the test. No information is available on whether, e.g. substances with a relative low bioaccumulation potential might have long-term effects on in-soil organisms.

At the moment, there is no validated trigger for bioaccumulation and potential effects on the soil ecosystem. In the ECHA guidance on PBT/vPvB assessment (ECHA 2014), it is concluded that for soil organisms no trigger can be set due to lack of data, but decision about bioaccumulation should be made on a case by case basis. However, the document also states that 'lipid and organic carbon normalised BSAF (biota to soil accumulation factor) values of 0.5 and higher are an indication of high bioaccumulation (ECHA 2014, page 119)'. Simon et al. (2016) showed that experimental BCF values for fishes are not well correlated to experimental BAF values for earthworms; thus it is not an option to extrapolate from aquatic to terrestrial organisms. Furthermore, it is unclear how the effects resulting from bioaccumulating substances should be addressed in the risk assessment for PPPs. Further research is needed, both aimed at the trigger for including bioaccumulation as for the methods to study the effects.

9.6.3. Avoidance

For a number of test organisms, avoidance tests are available, standardised or from literature (Table 36).

Table 36: Overview of avoidance test methods

Taxonomic group	Test species	Reference
Earthworms	*Eisenia fetida/Eisenia andrei*	ISO (2008)
Enchytraeids	*Enchytraeus albidus*	Amorim et al. (2008a,b)
Mites	*Oppia nitens*	Owojori et al. (2011)
Woodlice	*Porcellionides pruinosis*	Loureiro et al. (2005)
Springtails	*Folsomia candida/Folsomia fimetaria*	ISO (2011)

Avoidance tests are mainly used for retrospective risk assessment, e.g. the assessment of contaminated soils. Avoidance test are of relatively short duration (48 h), and thus give quick results. In a number of cases, the sensitivity of the avoidance test is compared with the acute toxicity data for a specific substance, and appears to be representative and thus suitable for assessing the contamination (e.g. De Silva and Amarasinghe, 2008; Römbke, 2008).

Avoidance tests are not part of the current risk assessment for PPPs. Some authors suggest that the avoidance test is sensitive and could be used as an alternative to an acute toxicity test (e.g. Garcia et al. (2008) for benomyl, carbendazim and lambda-cyhalothrin). Other authors, however, (e.g. Novais et al. (2010), based on results for phenmedipham, atrazine, carbendazim, pentachlorophenol, dimethoate and lindane) show that avoidance behaviour for *E. albidus* was not clearly correlated with survival and reproduction and do not recommend use of the avoidance test as an alternative to toxicity tests.

Results of avoidance tests can yield valuable information interpreting, e.g. effects found in long-term laboratory studies as compared to field studies. Moreover, assuming that avoidance response to a certain dose of a PPP is inversely related to the potential (re)colonisation of an area contaminated with that PPP dose, avoidance results can help to predict recolonisation of in-field areas (Renaud, 2012). If coupled with population models, avoidance tests can help in predicting spatially based recovery of

in-field populations (Meli et al., 2014). Avoidance tests might well play a role as an alternative for range-finding tests for chronic toxicity studies.

9.6.4. Biomarkers

Numerous studies with biomarkers are available. For an overview, see e.g. Kammenga et al. (2000) and Fontanetti et al. (2011). Biomarkers can be used as a sensitive indicator for exposure, but also to predict ecological effects. Information from biomarker assays could provide supporting information for risk assessment. In particular, they might be especially useful in the case that the standard tests do not show effects within the duration of the test but, due to the mode of action of the substance, delayed or long-term effects can be expected.

In an external EFSA report (Duncan et al., 2009), a review was made of long-term effects on invertebrates after short-term pulsed exposure. In that review, concerning in-soil organisms, only an example related to effects of diazinon on *Porcellionides pruinosus* is mentioned. Five weeks after application an effect on protein content occurred, that might result in effects on reproduction. It is not clear whether these effects would have been detected in the standard laboratory tests for prospective risk assessment.

9.7. (Semi)field methods for higher tier testing

9.7.1. Available (semi)field methods

Appendix K gives an overview of (semi)field studies evaluated by Brown et al. (2009) and Schäffer et al. (2010) with some additions from literature, but has no claim of being exhaustive.

In current regulatory field-test protocols, tests are performed in-field on replicated plots and study effects on 'natural' in-soil populations present at the time of the experiment. In other field-study methods, specimens were added to the system. In some cases, test systems are chosen on an uncontaminated soil (e.g. grassland), and the test substance is applied to the test soil or system. In the current procedure, laboratory tests are performed with arthropods, non-arthropods and microorganisms. Some field-study methods (e.g. the earthworm field study) are aimed at refining the particular first-tier test. In Section 4, it is proposed to study the natural assamblages of the soil community. It is clear from Appendix K that most field studies are aimed at effects on natural assemblages of the soil community.

Semifield studies are defined as controlled, reproducible systems that attempt to simulate the processes of and interactions between components in a portion of the terrestrial environment, either in the laboratory (small scale) or in the field, or somewhere in between. Single-species field studies with earthworms are submitted for the peer-review process in a standardised way.

The mobility of the test species in semifield studies is artificially limited, using e.g. enclosures or TMEs. TMEs can be conducted in the field, but it is also possible to move the TMEs to the laboratory and to study effects under controlled conditions.

Table 37: Main features of Terrestrial model ecosystems (TMEs) (Schäffer et al., 2010)

Guidelines	ASTM (1993), UBA (1994), USEPA (1996), Knacker et al. (2004)
Principles	Interaction of soil properties and the natural community of microorganisms, animals, plants
Species	Natural soil-organism community
Substrate	Undisturbed soils from field sites
Duration	Usually about 16 weeks
Parameter	Wide variety of fate and effect endpoints
Experience	Fungicides, contaminated field soil, or pharmaceuticals in dung

Schäffer et al. (2010) classify the different types of semifield studies according to a number of ecological and performance criteria (Table 38). The evaluation of the criteria was based on expert knowledge. They distinguish three main types of semifield studies: the assembled system, the terrestrial models ecosystem, and field enclosures.

Table 38: Rough classification of three semifield methods according to eight ecological and performance criteria. The grey shading indicates whether the criteria are fulfilled or not (from Schäffer et al., 2010)

		Assembled System	TME (TerrestrialModel Ecosystem)	Field enclosures
Ecological criteria	**Relevance**	Artificial food chain, but no real competitors/prey-predators	Natural community	Addition of organisms possible
	Endpoints	No community measures	All 'known' parameters can be measured	All 'known' parameters can be measured
	Flexibility	No crop simulation possible	Most soils, except very sandy/very dense soils	All soils
	Sensitivity			
	Practicability			
Performance criteria	**Reproducibility/repeatability**		Exact results not reproducible because of natural plasticity	Several studies, but few publications
	Experience	If similar approaches are combined	EU ring test	
	Standardisation	None	ASTM guideline and UBA draft available	IOBC guideline available

■ High/good/many
■ Medium/fair/numerous
□ Low/poor/few

As a result of the classification which was agreed at the workshop in 2007, Schäffer et al. (2008) conclude that TMEs are at promising method for assessing effects of pesticides.

Below the different types of studies are discussed in more detail.

Assembled systems

In assembled systems defaunated (sieved) soil is used, to which lab cultured organisms or parts of the natural community organisms are added, so that e.g. food chain effects can be studied. Their role is limited because *inter alia* it is hard to predict what happens when an additional species is added to the system. Studies with assembled systems can be very useful to answer specific scientific questions. Their role for risk assessment, however, could be in the intermediate tier B.

In the intermediate tier B of the proposed risk assessment flowchart (see Section 7.8 and Figure 13), an option is given to the applicant to provide (or to the risk assessor to require) additional information on effects to particular soil-organism groups of interest, gaining relevance by assessing mainly indirect effects of the substance of concern towards these groups.

The proposed test for this tier is a microcosm set up using natural, defaunated soil to which fragments of the natural community of the group of in-soil organisms of interest is added (e.g. microarthropods, nematodes, mesofauna including microarthropods and enchytraeids). These systems resemble the existing gnotobiotic tests (Schäffer et al., 2010), but have a major difference; instead of assembling the tested community with a limited number of fully known species, usually those existing in laboratory cultures, these microcosms contain a true fraction of the natural community collected in the field, thus increasing the number of species in the system and therefore the possible interactions between them (e.g. either via competition or predation).

In brief, samples are collected in the field, the natural community is extracted (by adopting standard methods for each organism group – see Römbke et al. (2006) for the sampling and extraction methods for different organism groups) and then it is added to the spiked natural soil on the microcosms. Depending on the group of interest, different methods can be adopted to decrease the variation on the number and composition of the community in each replicate microcosm.

Although the experiment is set up to focus on a specific group of in-soil organisms (e.g. microarthropods), species from other groups could be added to the system to increase the realism (e.g. nematodes or enchytraeids). Microorganisms are always included by re-inoculating the soil with a microbial suspension extracted from the same natural soil.

This means that the microcosm setup can be tailored according to data needs and to the type of substance. For instance, if focusing on an insecticide, the group of interest could be the microarthopods. This means that, although other species from other groups (e.g. enchytraeids) could be added to the microcosm to increase realism, only the microarthropods could be assessed at the end. Organisms can be identified at species or at life-form group level, or using any other trait-based typology of interest.

Besides having the advantage of working with a relevant fraction (in terms of number of species) of the natural community of interest, assessing not only direct effects to several species but also indirect effects via interactions among them, these systems have the advantage to ally the reproducibility of laboratory single species tests (mainly regarding the possible number of replicates) with a gain in ecological realism.

There is limited experience of assessing effects of chemicals, especially PPPs, using this approach. Effects of carbofuran have been assessed on nematode and microarthropod communities from temperate and sub-tropical soils by Chelinho et al. (2011, 2014). Data on nematodes showed not only the decrease in abundance and richness, but a shift in community composition of these organisms (although no shifts were detected on feeding groups). Data on microarthropods clearly showed that the direct effect on Collembola (an overall reduction in abundance and species richness together with shifts in community composition favouring epigeic over euedaphic species) clearly affected the abundance of competitor-mite species (an increase in oribatids was observed) and originated a decrease in the abundance of predatory mites.

While not aiming to replace a TME or a field study, where a higher level of ecological realism can be obtained, these test setups could help to obtain more realistic information on both direct and indirect effects on specific communities of interest with much less workload and a higher level of statistical power. Nevertheless, due to the limited experience available, further research is needed to refine some methodological aspects, namely on the size of the test vessels according to the community of interest, number of replicates, and the duration of the experiment to accommodate the variation in the life cycles between species.

Terrestrial Model Ecosystem (TME)

Table 39: Main features of Terrestrial model ecosystems (TMEs) (Schäffer et al., 2010)

Guidelines	ASTM (1993), UBA (1994), USEPA (1996), Knacker et al. (2004)
Principles	Interaction of soil properties and the natural community of microorganisms, animals, plants
Species	Natural soil-organism community
Substrate	Undisturbed soils from field sites
Duration	Usually about 16 weeks
Parameter	Wide variety of fate and effect endpoints
Experience	Fungicides, contaminated field soil or pharmaceuticals in dung

The experience with TMEs is increasing. Protocols are available from ASTM (1993), UBA (1994) and an updated protocol was presented at the PERAS workshop (see Section 2.2), see Table 39. Knacker et al. (2004) describe a number of TMEs exposed to carbendazim in different regions, comparing the results from the TMEs with those in the field. In his thesis, Scholz-Starke et al. (2013), describes the results of TME studies with lindane. The pros and cons of four types of TME (defined by Knacker et al. (2004)) are discussed: closed, homogenous TMEs; closed, intact TMEs; open, homogenous TMEs; and open, intact TMEs. TMEs are suited to studying effects on a number of invertebrate species, including some earthworm species. It is also possible to include effects on microorganisms, including functional endpoints, by e.g. including litterbags or bait lamina tests in the TME. Different aspects of TMEs correlated with their use as higher tier studies related to the risk assessment flowchart presented in Section 7.8, see also Figure 13, are discussed below in Section 9.7.2.

Field enclosures

Schäffer et al., 2010 define field enclosures as systems with undisturbed soil, where migration of species is prevented by barriers. Enclosure studies can focus on natural occurring communities, but also studies with added organisms where found. However, according to Schäffer et al. (2010), the added organism in practice are non-target arthropods living on the soil surface.

Field studies

Field studies without enclosures have been carried out with specific groups of organisms. Earthworm field studies are being carried out and used in the risk assessment since many years according to guidelines by BBA (1994), ISO (1999, 2014) and Kula et al. (2006). Field studies with in-soil microarthropods are being carried out since many years with a design based on the paper of Römbke et al. (2009).

Efforts are being made to address potential drawbacks of those field test (analysis of the statistical power of the field test, external recovery, dose–response design, etc.). For the ISO guideline for earthworm field studies, a revision is currently under way (SETAC GSAG/OECD expert group). Work on the standardisation of the in-soil microarthropod field study is also under way. Standardisation needs and options are also under discussion (Pieper et al., 2016).

Litter bag study

In the SANCO Guidance (European Commission, 2002), the litter bag method is always recommended in case of substances with DT_{90} higher than 365 days. The test is conditional for substances with a DT_{90} between 100 and 365 days and/or high risk is identified at lower tiers on soil fauna (earthworms, collembolans, mites) and microorganisms.

The method was considered an appropriate higher tier study at the time of the SANCO Guidance development since a wide range of in-soil organisms is involved in organic matter degradation.

According to the test design, litterbags containing dried organic material are buried in the soil of an arable field site which is treated with the test substance according to the representative uses reported in the good agricultural practice (GAP) table. The litter bags are sampled by removing from the soil after certain time periods using at least three sampling dates for a total duration of the test of minimum 6 months. With regard to the exposure, the annual rate (with crop interception) is applied on top of the plateau concentration, before the litter bags are buried. The mass loss of the organic material in the control and treatment groups of litter bags are determined for each sampling date as relevant endpoints. In addition, the breakdown (mass loss) rate between each individual sampling date

and between the start of the study and the last sampling date should be reported for the control and the treatment. The test is considered valid if at least 60% mass loss has occurred in the control plots at the end of the study (European Commission, 2002; OECD, 2006). In the risk assessment scheme, risk for soil organisms is considered acceptable if no significant effects on organic matter decomposition are detected at study end.

However, although recommended in the SANCO Guidance, the usefulness of this study design can be questioned. In the risk assessment proposed by the SANCO Guidance, a litter bag test is triggered either by a high risk identified in one of the single species test (earthworms, collembolans or mites) or by an effect on nitrate formation of more than 25% as proxy for the activity of soil microorganisms compared to control. In the case of both soil fauna and soil microorganisms, the litterbag test method is not considered appropriate as an higher tier approach for several reasons. Firstly, the link between the outcome of this test and the SPGs as defined in 6 is confounded by the fact that organic matter decomposition is performed jointly by microorganisms and soil fauna with different activity shares during the breakdown processes. Secondly, being an integrated measurement of activity litter biodegradation can be observed even if some species or functional group has been lost or their abundance has been highly reduced. Therefore, this test is not considered appropriate to refine risk to populations of soil organisms at the higher tier.

9.7.2. Addressing specific protection goal in (semi)field studies

9.7.2.1. Specific protection goals for in-soil fauna

Specific protection goals are proposed for in-soil organisms as drivers of particular ecosystem services, both in-field and off-field. When interpreting field studies, it needs to be made sure that they are able to address the SPGs and to detect relevant effects on the key drivers. The currently proposed specific protection goals for in-soil animals are (these may be adapted following the risk manager consultation):

In-field: For earthworms, enchytraeids, microarthropods, macrodetritivores (e.g. isopods), nematodes, molluscs (slugs and snails), small effects (10 < 35%) up to months on abundance/biomass of populations are tolerable.

For enchytraeids, microarthropods, macrodetritivores (e.g. isopods), nematodes optionally also medium effects (35 < 65%) up to weeks on abundance/biomass of populations are tolerable.

Off-field: For all organisms only negligible effects (< = 10% or NEL).

9.7.2.2. Statistical power to detect relevant magnitudes of effects in field studies

It is important to understand the power of various field study designs to detect effects at magnitudes relevant to the specific protection goals. Brock et al. (2015) develop a structured approach to the application of the MDD in the aquatic context and this may provide a basis for a similar development for in-soil organisms. For earthworms, in Section 3, it is indicated that in-field small effects on abundance and biomass (10–35%) for months are acceptable, off-field no effects (< = 10% or NEL) are acceptable. In the present earthworm field study according to ISO 11268-3 (ISO, 1999, 2014), with improvements of Kula et al. (2006), effects are assessed after 1, 6 and 12 months, however, the assessment endpoints recovery after 1 year. Often, only a limited number of dosages is used and, in the test design used, it is not possible in practice to detect effects of less than 50% on overall abundance and/or biomass with sufficient statistical significance. In more recent studies, it appeared possible to lower the MDD to 30–40% for total abundance (Vollmer et al., 2016). For individual species, which have lower abundances, the ability to detect effects will be lower. The standard endpoints of the test thus do not fit with the required level of protection. In the test protocol, it is also prescribed to measure effects after three and 6 months, indicating that potentially the duration of effects is in line with the data requirements. In the present design, however, the field test with earthworms will not be able to show whether effects of 10–35% occur, or to derive a NEL or an EC_{10}. An example how to calculate the power of the test is given in De Jong et al. (2006). This implies that it might be needed to adapt the study protocol, in order to obtain results that can be used to address the acceptability of effects identified for the SPGs. In general, presampling within a given field is essential to the study's evaluation.

In TME studies, the number of replicates can be higher; a dose response design can be followed, which means that it is more realistic to derive ECx values from the results, with a higher statistical reliability than in the case of field studies. Since the distribution of in-soil organisms in agricultural fields might be highly variable, variation in TME studies can also be high and the statistical power

should be checked. Earthworms can be tested in TME studies as well (Römbke et al., 2004). However, the variation appeared to be relatively high, so it is also questionable in this case whether TME studies with earthworms would be able to yield a reliable EC_{10} or EC_{35} value.

For nematodes in small-scale microcosms, the MDD was found to be < 20% (Höss et al., 2014). In field studies for collembolan MDD of ≥ 40% was found (Mack and Knaebe, 2016), Scholz-Starke (2013) conducted TME studies that on average were able to detect between approx. 5–10% (nematodes) and 40–50% (collembolans and enchytraeids) deviation from the control level. The MDD (minimal detectable difference) can be decreased by increasing the number of replicates. This can be the number of TME cores, but in the case of low numbers an option can be to increase the size of the cores so that the number of samples within the core (and thus the number of individuals) can be increased. MDDs can also be decreased by improving the sampling techniques (Brock et al., 2015). It should be elaborated how the sampling techniques can be adapted to be able to detect an certain level of effect.

In the case of experiments using natural communities of in-soil organisms (suggested as a surrogate reference tier), control and treatment communities will often have a different composition. If the differences in composition are potentially large, a large number of replicates will be needed to achieve a MDD less than 100%. Such differences can partially be accounted for by the Henderson and Tilton (1955) calculation or related methods. However, with such a method, it is not possible to obtain a statistical quantity like the MDD.

Until now, a power calculation has been performed for a limited number of the field protocols available for in-soil organisms. To be able to use the field protocols in regulatory practice, sufficient replication and abundance of relevant species should be ensured to be able to detect the above-mentioned magnitudes of effect.

9.7.2.3. Assessing long-term effects and studying recovery

At present the only field study that is widely used to study effects on in-soil organisms is the field study with earthworms. At present, recovery 1 year after application is taken as an endpoint; for further details see Section 2.

It is therefore recommended, for the time being, to study community recovery only experimentally in field studies. As noted earlier (see Section 7.4), recovery over long time periods may be best addressed by complementing experimental studies with population modelling, but impacts and recovery at community levels cannot be assessed using population models and therefore need to be assessed using field studies. As stated in Section 7.3, population modelling potentially may address (if sufficiently verified/validated) the impact and recovery from year on year application of PPP on in-soil organisms species in a system approach. Effects of PPP use and recovery at community levels cannot be assessed using population models for the time being and therefore need to be investigated in (semi) field studies with intact communities of in-soil organisms.

Depending on the fate and behaviour of the substance in soil, the application timing, the lifestage of the exposed organism as well as toxicokinetics and toxicodynamics, effects on the community might be detectable at later stages. This might be the case even if there are no effects visible in the short term or if recovery of short-term effects is observed. Long-term impacts may be related, e.g. to disrupted trophic interactions or to reproductive effects. Several species of in-soil organisms are univoltine, some may only be able to complete one generation after several years (please refer to Sections 3.2.1 and 7.9). Therefore, the timeframes for assessing impacts and recovery at community levels in (semi) field studies with in-soil organisms need to be appropriate to detect delayed effects of the application of a test substance. Effects may also take years to manifest. As reported by Pelosi et al. (2015) when comparing the effects of different cropping systems on earthworms over 15 years, it took more than 9 years for a reduction in earthworm abundance in conventional cropping systems to be detectable compared to organic cropping. Therefore, it should be ensured that experimental approaches are able to detect magnitudes effects that may only be visible after several years under variable field conditions (e.g. due to external recovery and diverging environmental conditions). This emphasises that experimental higher tier approaches need to be as controlled as necessary to be able to understand and predict long-term effects of pesticides.

As can be seen in the Figure 25, field sizes in Europe are mostly above 10 ha/field, in intensively managed areas up to 60 ha per field. It is likely that, considering the relatively low dispersal ability of in-soil organisms (see Section 3.2.2) and the average field sizes in Europe, recolonisation of fields by in-soil organisms from off-field within a year will be very limited. Experiments studying external and internal recovery in the same plots will yield only a very limited informative value as to whether SPGs are fulfilled.

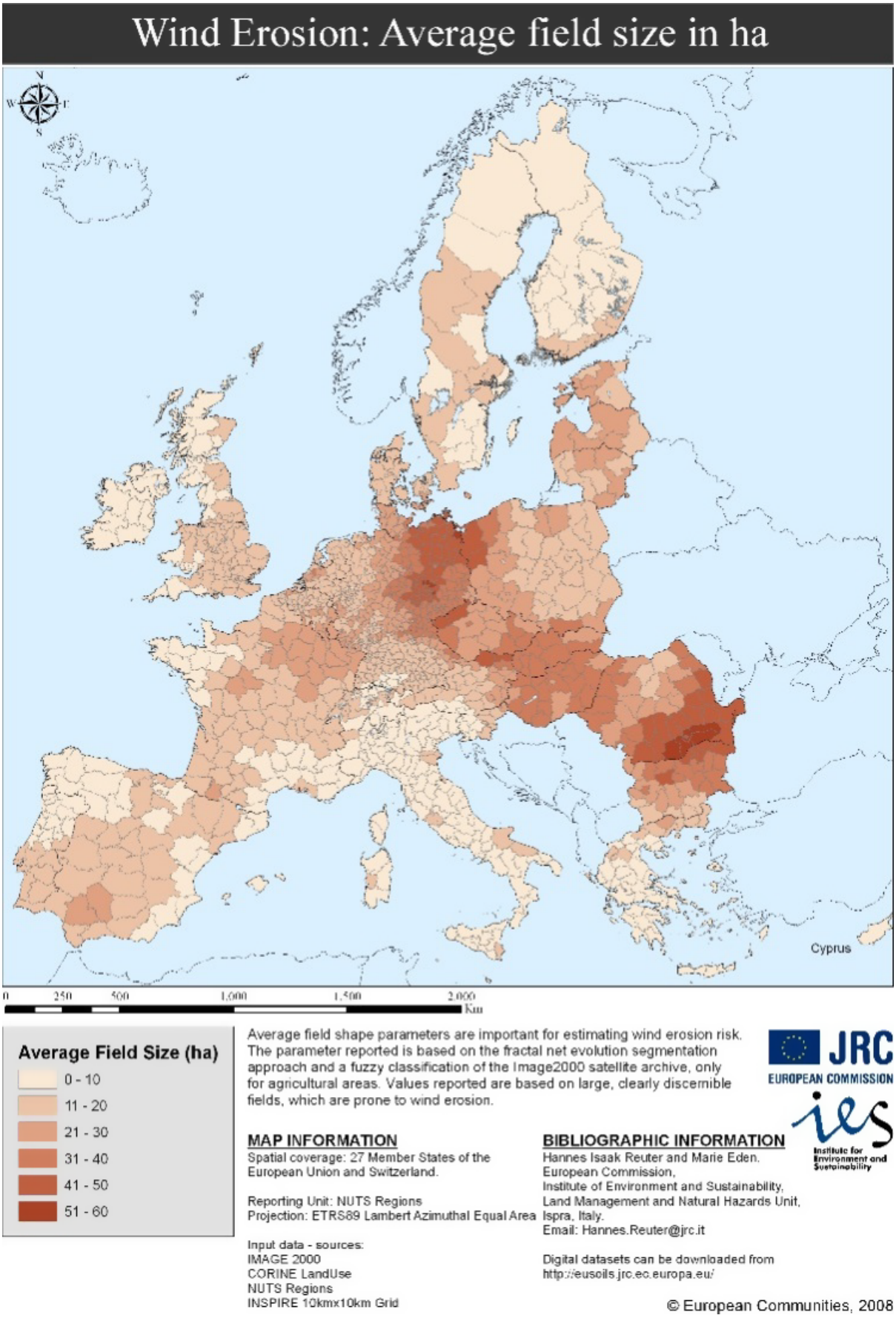

Figure 25: Average field sizes in Europe (see Reuter and Eden, 2008)

In the example of earthworm field studies performed according to ISO 11268-3 (ISO, 1999), plot sizes are 8 × 8–12 × 12 m and plots lie 2–5 m apart in a randomised block design. Considering the mean dispersal rates for different earthworm species (up to 10 m per year, see Section 3.2.2), it is very likely that significant migration between plots and the surrounding area will occur within a year, which will be the case to a much lower extent for the majority of European fields being mostly > 1000 times larger than study plots in earthworm field studies. Therefore, external recovery is likely to be severely overestimated in such studies.

A recent study by Ernst et al. (2016) aims to address recovery of Collembola in a multigeneration study testing *F. candida*. This approach does not, however, sufficiently address the properties of the types of potential stressors and the specific features of the landscape, i.e. variations in land use, and the types, spatial distribution and connectivity of habitats, as reported in Section 7.4 and is therefore, for itself, not considered suitable for assessing recovery (please refer to Appendix J).

In order to incorporate recovery in the risk assessment, it is important to keep in line with the specific protection goals. Protection goals are defined in Section 6, indicating an acceptable magnitude and duration of effects as well as the relevant spatial scale. On the basis of protection goals, focal taxa, focal communities and/or focal landscapes should be identified, based on relevant traits. For in-soil organisms, it is suggested that risk assessment is performed at the in-field scale (i.e. encompassing variation of factors that determine local differentiation of populations) and at field-boundary level (encompassing variation between in-field and off-field habitats representing); please refer to Section 6.1.

Scholz-Starke et al. (2013) conducted TME studies with lindane. Stability of the TMEs could be influenced by, e.g. effects of isolation, effects on diversity by predators, or removing of soil for sampling purposes. Scholz-Starke defined four criteria that should be met in order to ensure stability of the system: abundance, diversity, similarity and soil removal. The results show that TMEs are relatively stable, and therefore suited to detect the possibility for recovery, even for a relatively persistent substance as lindane, and for a recovery period of 1 year. This implies that the duration of a TME study is such that the majority of the taxa present will be able to show reproduction.

For field experiments to assess the risk at the field scale, it is suggested to choose a test design that excludes unrealistic external recovery (e.g. by surrounding the plot by a large enough strip of PPP treated area preventing that even animals with relatively high dispersal ability to recolonise the plot being assessed). This will allow a realistic worst-case prediction of long-term effects at the local scale.

It is possible to study the interaction at the field-boundary scale to determine the contribution of migration to recovery under realistic conditions. In order to be able to make generalised predictions, however, the information from field-boundary experiments needs to be integrated in the risk assessment.

9.7.3. Exposure in field studies

In both current regulatory field studies and proposed new methods like TMEs, the test substance is applied as described in the GAP and the concentration after exposure is measured in the soil. In earthworm field studies, analysis of initial soil concentrations is regularly performed following the recommendations of Kula et al. (2006). In TME studies, exposure is in general measured, often in separate cores (e.g. Knacker et al., 2004; Scholz-Starke, 2013). In contrast with laboratory studies, the substance is not mixed through the soil in order not to disturb the soil structure. Increasingly, a dose–response design is followed. Measurement of concentrations in combination with a dose–response design is needed to determine the consequences of risk-mitigation measures on direct, local PPP effects in off-field habitats or to help extrapolating to different in-field situations. In most standard lab studies, food is supplied after mixing the substance through the soil. Therefore, the uptake of the test substance via food cannot be quantified easily. In a field experiment, exposure via food is included by definition, since the in-soil organisms eat the food from the exposed environment.

9.7.4. Extrapolation/validation

In a large scale study at four locations in Europe, TME were conducted and results were compared with effects in the field (Knacker et al., 2004). Studies were conducted using carbendazim as the test substance. Functional endpoints were studied: nutrient cycling, soil-enzyme activity, microbial SIR and bacterial growth, feeding activity of in-soil organisms and organic matter decomposition. Although carbendazim did not result in clear effects for all parameters, and sometimes the variation between plots was rather large, the effects found were similar for the TME and the field situation for most

functional parameters (Förster et al., 2004; Sousa et al., 2004). As structural endpoints, nematodes, microarthropods, enchytraeids and lumbricids were studied. Although variability was again sometimes high, no differences were found between effects in TMEs and the field situation especially for nematodes (Moser et al., 2004a), enchytraeids (Moser et al., 2004b) and earthrowms, particularly on sites with anecic species as *Lumbricus terrestris* (Römbke et al., 2004).

When a valid field study is conducted, the uncertainty of its outcome with regard to the potential outcome under actual field situation should be assessed. Uncertainties that should be checked could be connected to, for example:

- product
- dosage
- method of application
- time, frequency and interval of application
- type of ecosystem (depends on abiotic factors as soil, climate and on composition of non-target groups)
- location and isolation of the test system
- region
- history of the test system
- crop and crop-stage
- in-field and off-field.

These aspects are discussed in detail in De Jong et al. (2005). Uncertainty could be addressed with an assessment factor. In order to determine the magnitude of the assessment factor it might be needed to conduct (semi)field studies under different conditions. Information from studies of, e.g. Dinter et al. (2013), describing the occurrence and distribution of earthworms in agricultural landscapes across Europe could help to determine the need to conduct (semi)field studies in different regions and/or the magnitude of the assessment factor for this aspect.

The assessment of the reliability of field studies and the interpretation of the effects is complex. It is important that field studies are reported in detail in a uniform way, to enhance a uniform assessment of the field studies. De Jong et al. (2010, 2006) describe in detail the assessment of higher tier studies for non-target arthropods and earthworms. These guidance documents helps in the assessment of the reliability of the field study as such, but also give guidance for the interpretation of the results. Such guidance does not exist for the other higher tier studies with in-soil organisms, but would be helpful for the interpretation of field-study results.

9.7.5. General recommendations for further development of existing field-study methods

- To characterise uncertainties with regard to exposure in field studies and to determine the worst-case character of exposure would require the measurement of the concentrations in the field study.
- Measurement of relevant concentrations in combination with a design with more than one dosage is needed to interpret the results and to determine, e.g. the consequences of risk-mitigation measures on direct, local PPP effects in off-field habitats or help extrapolating to different in-field situations. From a scientific point of view, a dose–response design would be ideal. From a practical point of view, this might be difficult and a compromise between number of replicates and number of dosages has to be found, comparable to, e.g. aquatic higher tier studies.
- It is important to understand the power of various field-study designs to detect effects and to evaluate recovery at magnitudes and time scales relevant to the specific protection goals. For interpretation of field experiments, not only representativeness but also vulnerability of the species present is important.
- Long-term field studies, over years, would be an important research priority. These would provide both validation for long-term impacts as assessed by population modelling, and indicate whether the population effects can be used as a surrogate for community effects. In addition, detailed data developed from long-term studies could be used to feed into food-web models to improve understanding of community interactions and energy-flows between functional groups (De Ruiter et al., 1998). In this way, food-web models may become tractable for predictive in-soil ERA.

Apart from field studies, there are other methods for refinement of lower tier uncertainties. One useful possibility is monitoring of actual in-soil populations in the field. Monitoring of the actual in soil population can be extremely helpful, especially considering the baseline level of population as reference control in the same field and areas where the substance should be applied. Publicly available databases, such as the Edaphobase (Germany) or monitoring programmes (e.g. the Netherlands Soil Monitoring Network, or the French 'Réseau de Mesures de la Qualité des Sols – Biodiversité') are important for setting the baseline conditions and to improve data evaluation. Long-term monitoring could be an important tool in the context of recovery evaluation and risk mitigation measures. For new substances, however, it is not really possible to monitor the long-term effects of the use of the substance in the field situation. For re-registration of existing products, where concern exists for in-soil organisms, monitoring data can provide useful information about the effects on the actual community in the field situation.

9.7.6. Recovery related to the specific protection goals

In all cases, whether an effect or the duration on an effect is acceptable requires consideration of the specific protection goal. It is important to differentiate whether the aim is to maintain biodiversity, or if the aim is to ensure the provision of a certain level of the ecosystem service (e.g. pest control, food-web support) by the identified drivers during a certain period of time. For example, for maintenance of biodiversity at field-boundary level the assessment could focus on whether external recovery from off-field habitats to in-field takes place. However, if the assessment aims at the protection of the drivers delivering a certain level of ecosystem services (e.g. food web support, pest control), then impacts may be unacceptable if they perdure too long even if the in-field community returns to its predisturbed state, it might at a much later time that does not allow for the timely provision of the services when they are needed. Therefore, when addressing recovery, also the time frame as part of the attributes of the proposed Specific Protection Goals has to be taken into account.

9.8. Testing metabolites

In line with the PPR Opinion on sediment organisms (EFSA PPR Panel, 2015b) and with the Aquatic Guidance Document (EFSA PPR Panel, 2013), the risk assessment to in-soil organisms of the relevant metabolites should be performed when they are formed or accumulated in the soil compartment. As reported in the Aquatic Guidance Document (EFSA PPR Panel, 2013), in order to minimise the testing, it may be possible to estimate metabolite toxicity by using non-testing methods, i.e. by identifying the presence of the toxophore (the active part of the molecule). It is noted, that the non-testing approach for deriving toxicity data would require valid QSAR models for the test species. If the potential effects of the metabolite are not sufficiently addressed by the effect assessment of the parent compound and the toxophore is still present in the metabolite, or it is unclear if the toxophore is present in the metabolite, then specific toxicity tests with the metabolite on the relevant test species (most sensitive with the parent) should be performed. If an assessment indicates that the toxophore is no longer present, in the first instance, it may be assumed that the toxicity of the metabolite is equal to the toxicity of the parent compound for all first-tier test species.

9.9. Modelling approaches to Surrogate Reference Tier and Recovery

Currently, the biological level at which the model should be targeted is the population. As can be seen from the general framework (see Figure 13 and Section 7.3), population modelling is considered to be an evaluation that integrates the effects of multiple exposures over time into the lower tier tests. Since recovery is an integral part of the population response, once we apply the systems approach we no longer need to consider long-term recovery explicitly, since if this does not occur, the population will decline. However, for some SPGs it might be necessary to consider short-term recovery, e.g. to maintain a ecosystem service needed at shorter time-scales.

Which type of model is appropriate for the systems approach to population modelling will depend on what data are available, and the modelling question. For in-soil organisms, spatial dynamics are not considered as being important, hence a greater range of model types can be considered than would be the case should action at a distance be important in the assessment.

Depending on model formulation, the model can be generic or specific to, e.g. a given species type, chemical type, landscape type. The domain of applicability of the model, including the extent of acceptable extrapolations, should be described by the modeller (EFSA PPR Panel, 2014b).

Since population modelling is considered to be an integration of multiple exposures over time into the lower tier tests, refinement of the models is permissible only by refinement of the exposure. This strategy is designed to prevent the population modelling becoming highly complex from a regulatory perspective by limiting its use to the preselected species and scenarios used for tier 1. This means that the models will need to be designed to work with the standard data from the submission dossier as the inputs, and only these values will be modified by the user, i.e. users will not be able to modify the ecological processes or parameter values for behaviour and ecology of the chosen species.

9.9.1. Overview of different types of effect models for populations

The types of model used in population-level risk assessment have been classified and reviewed, e.g. by Munns et al. (2008) and briefly by EFSA PPR Panel, (2014b). Here, we give only an outline summary. There are three main types of model in use; these are: scalar (unstructured), structured (e.g. matrix) and individual-based modelling (IBM, also known as agent-based modelling (ABM)). The model types differ in the degree of detail in the representation of the population and processes that can be represented.

Scalar models – These are the simplest form in which all individuals are treated as identical. They do not differ in age or in any other characteristic and so the population can be fully represented by a single scalar variable representing population size. Density dependence can be incorporated in scalar models, but in general they lack the necessary descriptive power to cope with multiple applications that might affect different stages of an animal's life-cycle differently.

Structured-population models – these are often implemented as matrix models. Distinction can be made between individuals in different categories, e.g. ages, sizes. The population is divided into age or size classes (e.g. juveniles and adults) and this gives the population structure, its structure being described by the numbers in each class. All individuals within an age or size class are treated as identical, but there may be variation between the classes in their survival or reproductive rates per unit time. The characteristics of the individuals in each class are entered into the cells of a matrix, and this allows computation of how population structure changes as time progresses. Models of this form can be quite complex.

Individual-based models – In individual-based models (IBMs or ABMs), each individual animal in a population is modelled separately. Individuals may interact with their environment (e.g. depleting food resources by feeding) and with each other (e.g. predation, reproductive behaviour). Individuals should differ in their characteristics, such as their age and size and energy reserves, and each acts according to its modelled needs, e.g. for food, or a mate, or to care for its offspring.

9.9.2. Considerations of models used for in-soil populations

Scalar models are probably too simple to be employed to model a system with complicated, temporally variable inputs and differential effects on life-stages, but structured population models will often be suitable for this purpose. If complex, these models do suffer from issues of mathematical tractability, but require fewer parameters and therefore fewer explicit assumptions compared with IBMs. Model-development time is also shorter. These models would therefore be most suitable in cases where general assumptions need to be made in the face of uncertain data. A further advantage is that, since these models are mathematical rather than logical, uncertainty is also easier to quantify.

IBMs provide the most detailed description of the effects of chemicals on populations, and generally use more parameters than other model types. The advantages of IBMs are that they can represent the effects of chemicals applied in environments that may change seasonally and can incorporate feedback loops and therefore include more complex ecological interactions than is possible with other model types. IBMs therefore provide the richest potential for prediction if the relevant mechanisms are included (Topping et al., 2015b). IBMs do not suffer from mathematical tractability issues, but they do require greater input of resources to development and testing than the other model types. IBMs have a further distinct advantage in that they can incorporate TK/TD models directly.

Toxicokinetic/toxicodynamic models (TK/TD models) – these are not population models but work at the individual level. In these models, the uptake of a chemical and its distribution between the organs of the body and its subsequent biotransformation and elimination processes are collectively referred to as toxicokinetics. Also, modelled are the effects of the chemical where it causes harm within the body, with consequences on individual performances and or life-cycle trait values, referred to as toxicodynamics.

These models can be used to refine an exotoxicological assessment when exposure over time is considered variable and important (e.g. in the case of vertical movement in earthworms).

9.9.3. Issues arising in selection of modelling focus

Depending on the species and spatiotemporal scales selected the modelling approach may vary considerably. For example, Reed et al. (2016) illustrate the use of two rather different soil organism models (for earth works and Collembola), each addressing different specific characteristics. Prior to making a decision on species and scales it is not possible to recommend details of the modelling approach, therefore the Panel provides general guidelines only.

Selection of species – there is no *a priori* reason for selection of one species over another in terms of population modelling. Therefore, species selection should be justified in terms of representativity and expected vulnerability based on demographic and ecotoxicological traits. The following demographic traits are relevant to the assessment of population recovery (Rubach et al., 2011):

- Life span;
- Survival to reproduction;
- Generation time (i.e. the interval between reproductive events);
- Voltinism (i.e. the number of reproductive events per year);
- Number of offspring (i.e. clutch size per reproductive event).

In addition, the ecotoxicological components of life-stage sensitivity and methods of incorporating multiple and long-term exposure need to be considered.

Spatial and temporal variation – for both the surrogate reference tier models and recovery, it is necessary to have a realistic, worst-case scenario of exposure during time and space. Since we do not consider action at a distance to have an appreciable influence on the assessment, spatial dynamics may not be necessary in most cases. However, it should be noted that spatial variation is scale dependent, and in the case where the in-field pattern of toxicant causes spatial heterogeneity at a scale commensurate with driving population processes potentially altering population dynamics, then this should be considered by the model (e.g. Meli et al., 2013). In the case of vertical movement, however, the same argument applies to in-soil organisms as to NTAs in space (see EFSA PPR Panel, 2015a), and integration of exposure and effects may need to be a dynamic modelling process rather than a combination of statistical distributions. In this case, TK/TD modelling will be needed. In order to maximise usefulness by integration with long-term factors and to prevent creation of further parallel tests, however, the TK/TD modelling should be integrated into population models.

9.9.4. Model development

Model development should follow the guidelines given by EFSA PPR Panel (2014b). This involves the following steps implemented in a modelling cycle, which is repeated until the model is considered to perform satisfactorily compared to predefined criteria (adapted from EFSA PPR Panel, 2014b):

1) The problem formulation sets the scene for the use of the model within the environmental risk assessment. It therefore needs to explain clearly how the modelling fits into the risk assessment and how it can be used to address protection goals. In all cases, for the evaluation of in-soil population impacts, the critical issue is the development of a baseline model that represents the state of the population under normal, realistic worst-case conditions, but without the stressor to be evaluated. Evaluation of the model will initially be based on the baseline, and only later on the implementation of the regulated stressor.
2) Model formulation. Based on the problem definition, a conceptual model is designed. The conceptual model provides a general and qualitative description of the system to be modelled. It characterises the environmental and biological processes and their interactions and interdependencies.
3) Model formalisation. In this step, model variables and parameters are defined and linked together into mathematical equations or algorithms. The result of this step is called the formal model.
4) Model implementation. In the following step, the formal model is transferred into a computer model by implementing the model equations into computer code. The computer code should be verified to check if it correctly represents the conceptual and formal model.

5) Model set up. In this step, model parameter values are estimated and the computer model is combined with one or more environmental scenarios. The result is called the regulatory model. Note that the regulatory model includes both the computer model and the environmental scenarios. Model analysis, including sensitivity analysis, uncertainty analysis and comparison with observed data, is an essential part of the procedure to set up the regulatory model.

In all steps, documentation of the procedure should be provided, and both the model and the computer code or mathematical equations should be documented and explained clearly for risk managers.

9.10. Mixture Toxicity

As a consequence of simultaneous (tank mixtures) or sequential applications of PPPs, in-soil organisms, like any other organisms in the environment, could also be exposed to mixtures of biologically active compounds.

Solutions which allow the assessment of simultaneous exposure to different pesticides are proposed in other EFSA opinions, such as (EFSA, 2013; EFSA PPR Panel, 2013, 2014a) and therefore not repeated in this Opinion. The Panel generally recommends the use of the concentration addition model to address mixture toxicity (EFSA PPR Panel, 2015a).

10. Conclusions and recommendations

10.1. Conclusions

The Panel has proposed SPG options to be considered in the risk assessment of in-soil organisms exposed to PPPs. Key driver organisms in soils are bacteria, mycorrhiza and other fungi, nematodes, earthworms and enchytraeids, microarthropods, gastropods and macroarthropods. The Panel has identified those ecosystem services that are important in agricultural landscapes and are provided by key-driver, in-soil organisms. These ecosystem services are the provision of genetic resources and biodiversity, the maintenance of cultural services, nutrient cycling, pest and disease control, natural attenuation of xenobiotics and toxins, the formation of soil structures and water-retention capacity, and the support of food webs in agricultural landscapes.

The Panel proposes different SPG Options for in-soil organisms living in-field and in off-field areas. Options are discussed for the magnitudes of in-field effects that can be suffered by key drivers without severely compromising the ecosystem-service performance. SPG Options are specific for each key driver group. This is because species traits affect the exposure and the sensitivity of the organisms towards the PPP and also the degree of recovery after disturbance.

With regard to persistent substances, the Panel considers that the SPG option proposed in Section 6 should also cover those situations. However, at the stage of developing the guidance document, it is recommended to do some example calculations with relatively persistent PPPs in soil in order to explore whether an additional assessment is needed for persistent PPPs.

Exposure assessments for in-soil organisms are performed according to the EFSA Guidance Document for predicting environmental concentrations in soil (EFSA, 2016). Scenarios are available for the three regulatory zones in accordance with Regulation EC No 1107/2009 of the European Parliament and the Council. Exposure assessments consist of five tiers for which user user-friendly software tools have been developed. The purpose of the exposure assessment is to consider the total area of the crop where it is intended that the PPP should be applied. In order to account for differences in the spatial statistical distribution of crops, scenario-adjustment factors were defined to correct the PEC calculations if necessary.

The soil-exposure scenarios are currently limited to annual field crops under conventional and reduced tillage. However, scenarios for the selection and parameterisation of scenarios for permanent crops and row crops on ridges are under development and will come available soon. Data on the litter layer are scarce and it is assumed that no litter layer, in the sense of a conventional soil horizon, is present in the majority of permanent crops. Grassland or bare soil scenarios are appropriate here. Litter layers might, however, become more important in the future; good soil-management practices are promoting an increase in organic matter on the soil, so there may be a shift to a more sustainable management of this litter layer.

The presence of PPPs on off-field non-target surfaces is mainly caused by the emission of the applied product out of the field by spray drift and run-off. Drift is currently considered to be the most

important factor for off-field emissions to non-target surfaces. Drift is defined as droplet drift, but vapour drift and dust drift are also considered to be important emissions in particular cases. The basic processes for run-off emissions were described by FOCUS (FOCUS, 2001). The methodology was originally developed for surface waters but it can be transferred to off-crop areas as described by EFSA PPR Panel (2014a). Several data sets exist for deposition of drift and dust and exchange of air-borne substances with receptor surfaces. These data sets should be combined in order to produce harmonised approaches (e.g. drift curves).

For soil microorganisms, a number of methods exist to measure soil microbial activity as function and structure. A number of interpretation problems are identified, i.e. effects on functional endpoints might be diluted by functional redundancy and for effects on structural endpoints the interpretation in the sense of their impact on the functions. Novel methods are under development in order to be able to measure a broad spectrum of functional and structural endpoints. These methods need further development and adjustment for use in risk assessment of pesticides, but are promising tools for the near future.

The intermediate tier B of the proposed risk assessment flowchart comprises a microcosm experiment with a fraction of the natural community. An option is given to the applicant to provide (or for to the risk assessor to require) additional information on effects of PPPs on particular in-soil organisms of interest, assessing mainly indirect effects of the substance on these groups. In order to address this subject, a microcosm setup is proposed, using natural, defaunated soil to which fragments of the natural community of the group of in-soil organisms of interest is added. Due to the limited experience available, further research is needed to refine some methodological aspects, and also to the interpretation of the results for risk assessment.

The use of TMEs is suggested as a most promising method for the surrogate reference higher tier, next to field tests, see also Section 7.8. As indicated in Section 9.7.4, in a study involving four different European sites comparing TME data and field data both fate and effect data showed that not only TMEs were able to mimic the variability of the data found in the field, but also showed comparable response patterns and magnitude of response (mostly measured as EC_{50}) within the same order of magnitude than those obtained in the field in most cases (Knacker et al., 2004). These and other TME studies mentioned in this opinion showed that TME experiments can be designed by using TMEs with different sizes and by having different number of replicates (or replicated samples within each TME) as such that they can show the desired level of magnitude and duration of effects. Since TMEs can last and remain stable for up to 1 year, they will also be able to detect effects on reproduction of the majority of the species. Evaluation of recovery of in-soil organisms' species requires a systems approach at the population level to determine the effect of a PPP on the population. This is because assessment needs to take into account the state of the population before exposure to the PPP, as well as long-term and multiple application effects on the population. Spatial dynamics are not thought to be generally important for recovery of in-soil organisms in-field. Since recovery is an integral part of the population response, the application of the systems approach to population modelling removes the need to consider long-term recovery explicitly; if recovery does not occur, the population will decline. For some SPGs, however, it might be necessary to consider short-term recovery, e.g. to maintain an ecosystem service needed at shorter time-scales. Functional tests, like litter bag test, are not considered appropriate to address the SPG options as described in Section 6 and refine risk to populations of soil organisms, since redundancy can occur and being an integrated measurement of activity litter biodegradation can be observed even if some species or functional group have been lost or their abundance has been highly reduced.

10.2. Recommendations

The Panel has identified Service Providing Units (SPUs) for different ecosystem services. The most critical SPU-ecosystem service combination has to be selected for the final SPG option. For ecosystem services that address structural parameters, e.g. biodiversity, the Panel has identified the *populations* of different species of in-soil organisms as SPUs (invertebrate SPUs). For ecosystem services based on soil processes, e.g. nutrient cycling, the Panel has identified as SPUs the abundance and biomass of different *functional groups* of in-soil organisms (microbial SPUs). Defining SPUs merely at the level of functional groups, however, may lead to a loss of functional performance under unfavourable conditions. In order to support the long-term functional role of in-soil organisms in agricultural soils, it is recommended to define the SPU as the abundance/biomass of populations of the species that make up the different functional groups.

In order to link exposure and effects reliably, the Panel recommends measuring the exposure concentration in laboratory tests and in field-test systems. Exposure could be measured using the two-

step extraction procedure that is proposed in EFSA (2016). This consists of a 24-h extraction with a 0.01 M $CaCl_2$ solution to characterise the pore-water concentration and a solvent extraction to characterise the total extractable mass. Measurements should be performed so that it is possible to characterise adequately the maximum concentration in both space and time. The Panel does not recommend calculating the pore-water concentration from the concentration in total soil using the partitioning coefficient. The Panel considers the use of calculated pore-water concentrations acceptable only for legacy studies where only the nominal concentration is available. It is now common practice to divide the obtained toxicity endpoint of lipophilic substances by a factor of two. The Panel considers that scaling of the toxicity endpoint is only justified when only the nominal concentration is available (legacy studies) and when the ERC is expressed in terms of a pore-water concentration. In all other cases, scaling of the toxicity endpoint is not justified.

A tiered approach to assessing the effects of PPPs on in-soil organisms should include a relatively simple, robust set of tests as the lowest tier. After reviewing the existing tests available, the Panel recommends carrying out tier 1 toxicity tests on 5 species/process.

The Panel recommends that further research should be conducted on the sensitivity of test species and tested exposure routes with the aim of developing improved test systems for tier 1 assessment. This is particular relevant for non-arthropod invertebrates where currently no robust conclusion can be drawn on suitable test systems to close existing gaps and on assessment factors.

Exposure *via* food uptake is only partly included in the standard laboratory tests, since uncontaminated food is normally provided following exposure. When food is likely to be an important exposure route, the Panel recommends adaptation of the test protocol for a better estimation of the toxicity due to oral uptake. In particular, the Panel recommends the design of test protocols for carnivorous, non-target, in-soil invertebrates to account for possible trophic chain effects, such as biomagnification. Until new tests are developed, it is recommended to adapt the test with *H. aculeifer* in order to take exposure via food into account, so as to address properly the exposure of predators.

Isopods are key drivers for several ecosystem processes and the most important route of exposure for most isopod species is feeding on litter debris. Therefore, the panel recommends development of a standardised test addressing both feeding and reproduction parameters, to assess effects on key in-soil organisms of exposure to PPPs via consumption of litter debris. The available information on isopods makes an isopod test a good candidate for developing an ISO or an OECD guideline.

Since the present test aimed at N transformation covers a number of processes, it is considered a relevant indicator, at least for the ecosystem services nutrient cycling and food-web support. In the present test design for agrochemicals, it is prescribed to test two dosages, and to determine at different points in time whether effects on nitrate formation rate are > 25%. When effects exceed 25% after 28 days, the test can be prolonged to 100 days. For non-agrochemical substances, however, a dose–response design is prescribed. In order to assess whether the effects fit with the magnitude and the temporal scale mentioned in Sections 4 and 6, it is recommended to use a dose effect design for agro-chemicals as well.

The present standard test species do not include mycorrhizal fungi. Mycorrhizal fungi are considered very important for many ecosystem services but they are not covered by the N-transformation test. The ISO test with *Funneliformis mosseae* (formerly *Glomus mosseae*) allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design. It is therefore recommended to develop an additional standard test with mycorrhizal fungi.

Considering the possibilities for intermediate tier testing, the panel considers that the SSD conceptual model is very useful (in intermediate tier A), but that standard SSD methodology cannot yet be applied to in-soil organisms until further guidance will become available on the combination of data for in-soil organisms in SSD.

In the current risk assessment for in-soil organisms exposed to PPPs, the different groups of in-soil organisms are assessed separately, both at lower tiers and at higher tier assessment steps. As a consequence, the evaluation of indirect effects, e.g. via food-web interactions, is not possible. In order to overcome these limitations and to fulfil the legal requirements, the Panel recommends assessment of the response of *communities* of in-soil organisms to intended uses of PPPs. The assessment of the in-soil organisms' community response can be performed at higher tiers by investigating the effects of PPPs in tests with natural assemblages of in-soil organisms, e.g. in field tests or in terrestrial model ecosystems (TMEs). In the calibration of lower tier assessment steps, direct and indirect effects of PPPs on the community of in-soil organisms should be taken into account in order to derive appropriate assessment factors. Currently, only the earthworm field study is performed on a regular basis. In the risk assessment scheme proposed here, it is recommended to test soil communities in the

higher tier, therefore the sole performance of an earthworm field-study test may not be appropriate any more. The panel also recommends use of TMEs as a tool to study effects on soil communities in the (semi)field. Further development of the TME method into an ISO or OECD guideline is recommended.

The Panel proposes that population modelling is used to integrate long-term and multiple stressor events into the population-impact evaluation as a lower tier test. The assessment should be based on a number of standard models of species identified as being potentially vulnerable to these effects. Since standard models are proposed, the Panel recommends that modification by users should be limited to PPP properties and GAP scenarios.

The Panel recommends taking into account the recommendations of the draft EFSA guidance on Uncertainty in Scientific Assessment when developing guidance for standardised risk assessment for in-soil organisms. In particular, it is recommended that uncertainties affecting the standardised assessment procedure should be systematically identified and described and that as many sources of uncertainty as possible should be included transparently in proposed assessment factors.

The Panel recommends that assessment factors be derived probabilistically on the basis of statistical modelling of the relationships between effects for different species in the various possible lower tier tests, higher tier studies and the surrogate reference tier. In particular, a Bayesian graphical model can exploit information from both experimental data and expert judgement and provides a relatively transparent method for deriving assessment factors in order to ensure high probability of acceptable effects for uses that pass the risk assessment.

10.3. Specific recommendations for further research

- Most laboratory test methods are designed for spray applications, but should be adapted and in order to test effects of soil fumigants, treated seeds and granules.
- In current laboratory test systems, in-soil animals are provided with uncontaminated food. It is recommended to develop or to amend laboratory test systems that integrate both contact and oral exposure routes.
- In current laboratory test systems, in-soil fauna is exposed mostly to standard artificial soils with high content of organic matter as peat. The Panel recommends that further research elucidates which parameter modulate toxicity of PPP in natural agricultural soils, in order to better extrapolate from the lab to the field situation. When possible, natural agricultural soil should be proposed and used in standard laboratory testing.
- The MicroResp® method measures respiration rate in, e.g. soil and can describe the capacity of microbial communities of soil to degrade various organic substrates. It is recommended that the capacity of MicroResp® to study effects of pesticides on soil microbial processes is determined in future research.
- The Panel recommends adding a test with mycorrhizal fungi. The ISO test with *Funneliformis mosseae* (formerly *Glomus mosseae*) allows for adaption of the endpoints measured or to test other species/strains. Further research and development is needed to improve the test design in these respects.
- The Panel recommends development of a standardised test with isopods addressing both feeding and reproduction parameters, to assess effects on key in-soil organisms resulting from exposure to PPPs via consumption of litter debris.
- There is only limited experience of combining toxicity data of in-soil organisms in SSD, especially for PPPs. Further research is needed with regard to combining toxicity data from different groups of in-soil organisms. This further research is aimed at making (chronic) toxicity data available in general, and the role of the toxic mode of action of a PPP and the identification of sensitive groups in particular.
- Mesocosm data for many more active substances are needed to make possible calibration of lower tiers against the Surrogate Reference Tier; calibration would then be based on data rather than on expert judgment alone.
- Research to develop data to support the use of TK/TD modelling for organisms where vertical movement in soil may require dynamic linking between exposure and effects.
- Exposure profiles vary in both time and space. Toxic effects of PPPs in soil organisms should be best linked to internal body concentrations. For the time being, not enough data are available on TK/TD of PPPs in soil organisms. For these reasons, the following research needs are proposed:

  - Reliable models of movement for endogeic earthworms, within the soil profile;
  - Dynamic models of exposure providing soil and pore-water concentrations at all relevant soil depths and varying with time;
  - TK/TD models capable of linking toxicological effects to internal body concentrations in time;
  - Ideally, these three combined components would be integrated into the system model used to develop the population-modelling 'surrogate reference tier'.

- Long-term field studies, over years, are an important research priority. These would provide both validation for long-term impacts as assessed by population modelling, and indicate whether the population effects can be used as a surrogate for community effects. In addition, detailed data developed from long-term studies could be used to feed into food-web models to improve understanding of community interactions and energy-flows between functional groups (De Ruiter et al., 1998). In this way food-web models may become tractable for predictive in-soil ERA.
- There is a need to develop a range of representative scenarios and models of relevant taxa for population modelling. These will need to include a definition of the normal operating range for the focal species, a parameterisation of the models, and incorporation of ecotoxicology in the models, and the range of system drivers needed (e.g. weather, regulated and non-regulated stressors). These models and scenarios will need to cover the range of agricultural systems necessary to provide good coverage for any regulatory ERA that may arise.

References

Abd-Alla M, Omar S and Karanxha S, 2000. The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes. Applied Soil Ecology, 14, 191–200.

Agerer R, 2006. Fungal relationships and structural identity of their ectomycorrhizae. Mycological Progress, 5, 67–107.

Aguilar-Trigueros C, Powell J, Anderson I, Antonovics J and Rillig M, 2014. Ecological understanding of root-infecting fungi using trait-based approaches. Trends in Plant Science, 19, 432–438.

Aislabie J and Deslippe J, 2013. Soil microbes and their contribution to soil services. In: *Ecosystem services in New Zealand: conditions and trends*. Manaaki Whenua Press, Lincoln, New Zealand, 143–161.

Akhurst R and Smith K, 2002. Regulation and safety. In: Gaugler R (ed). *Entomopathogenic nematology*. CABI Publishing, Oxford. pp. 311–332.

Aldenberg T and Jaworska JS, 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicology and Environmental Safety, 46, 1–18.

Alikhan MA, 1995. Terrestrial Isopd Biology. Ed. Alikhan MA.

Amador J, Gorres J and Savin M, 2006. Effects of *Lumbricus terrestris* L. on nitrogen dynamics beyond the burrow. Applied Soil Ecology, 33, 61–66.

Amorim MJB, Novais S, Römbke J and Soares AMVM, 2008a. Avoidance test with *Enchytraeus albidus* (Enchytraeidae): Effects of different exposure time and soil properties. Environmental Pollution, 155, 112–116.

Amorim MJB, Novais S, Römbke J and Soares AMVM, 2008b. *Enchytraeus albidus* (Enchytraeidae): a test organism in a standardised avoidance test? Effects of different chemical substances. Environment International, 34, 363–371.

Anderson J, Ineson P and Huish S, 1983. Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic-matter from deciduous woodlands. Soil Biology and Biochemistry, 15, 463–467.

Anderson JM, 1988. Spatiotemporal effects of invertebrates on soil processes. Biology and Fertility of Soils, 6, 216–227.

Anderson JM, 2000. Food web functioning and ecosystem processes: problems and perceptions of scaling. In: Coleman DC and Hendrix PF (eds). *Invertebrates as Web-Masters in Ecosystems*. CABI Publishing, New York, NY, USA. pp. 3–24.

Anderson RH, Prues AG and Kravitz MJ, 2010. Determination of the biologically relevant sampling depth for terrestrial ecological risk assessments. Geoderma, 154, 336–339.

Anzuay MS, Luduena LM, Angelini JG, Fabra A and Taurian T, 2015. Beneficial effects of native phosphate solubilizing bacteria on peanut (Arachis hypogaea L) growth and phosphorus acquisition. Symbiosis, 66, 89–97.

Arsel M and Buscher B, 2012. Nature (TM) Inc.: changes and Continuities in Neoliberal Conservation and Market-based Environmental Policy. Development and Change, 43, 53–78.

ASTM (American Society for Testing and Materials), 1993. Standard guide for conducting a terrestrial soil-core microcosm test, E 1197-87. In: *Annual Book of ASTM Standards, Environmental Assessment, Risk Management and Corrective Action; Biological Effects and Environmental Fate, 11.06*, 546–557.

Auclerc A, Ponge JF, Barot S and Dubs F, 2009. Experimental assessment of habitat preference and dispersal ability of soil springtails. Soil Biology & Biochemistry, 41, 1596–1604.

Baas-Becking L, 1934. Geobiologie of inleiding tot de milieukunde. Diligentia Wetensch. Serie 18/19, van Stockum's Gravenhange, 263 pp.
Bailey D, Held D, Kalra A, Twarakavi N and Arriaga F, 2015. Biopores from mole crickets (*Scapteriscus* spp.) increase soil hydraulic conductivity and infiltration rates. Applied Soil Ecology, 94, 7–14.
Baird DJ, Baker CJ, Brua RB, Hajibabaei M, McNicol K, Pascoe TJ and de Zwart D, 2011. Toward a knowledge infrastructure for traits-based ecological risk assessment. Integrated Environmental Assessment and Management, 7, 209–215.
Bakker F, 2016. Design and analysis of field studies with bees: a critical review of the draft EFSA guidance. Integrated Environmental Assessment and Management, 12, 422–428.
Barker G, 2001. *The biology of terrestrial molluscs*. CABI Publishing, Oxon, UK. p. 558.
Barker G, 2004. Natural enemies of Terrestrial Molluscs. CABI International, 645 pp.
Barnard RC, 1990. Some regulatory definitions of risk: interaction of scientific and legal principles. Regulatory Toxicology and Pharmacology, 11, 201–211.
Barrios E, 2007. Soil biota, ecosystem services and land productivity. Ecological Economics, 64, 269–285.
Barros E, Curmi P, Hallaire V, Chauvel A and Lavelle P, 2001. The role of macrofauna in the transformation and reversibility of soil structure of an oxisol in the process of forest to pasture conversion. Geoderma, 100, 193–213.
Bauer C and Römbke J, 1997. Factors influencing the toxicity of two pesticides on three lumbricid species in laboratory tests. Soil Biology & Biochemistry, 29, 705–708.
BBA (Biologische Bundesanstalt), 1994. *Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, Nr. VI, 2-3, Auswirkungen von Pflanzenschutzmitteln auf Regenwürmer im Freiland*. Saphir-Verlag, Ribbesbuttel, Germany.
Bedos C, Cellier P, Calvet R, Barriuso E and Gabrielle B, 2002. Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: overview. Agronomie, 22, 21–33.
Begon M, Townsend C and Harper J, 2006. *Ecology: from Individuals to Ecosystems*. Blackwell Publishing, Oxford.
Behan-Pelletier V, 1999. Oribatid mite biodiversity in agroecosystems: role for bioindication. Agriculture Ecosystems & Environment, 74, 411–423.
Beijerinck M, 1913. *De infusies en de ontdekking der bakterien*. Jaarboek van de Koninklijke Akademie van Wetenschappen, Muller, Amsterdam, pp. 1–28.
Belden JB, Gilliom RS, Martin JD and Lydy MJ, 2007. Relative toxicity and occurrennce patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean production. Integrated Environmental Assessment and Management, 3, 90–100.
Belfroid A and Van gestel CAM, 1999. *Blootstellingsroutes van toxische stoffen voor terrestrische invertebraten – literatuur studie*. Rapport E-99/06. Vrije Universiteit Amsterdam, Instituut voor Milieuvraagstukken, Amsterdam, The Netherlands.
Belfroid A, Meiling J, Sijm D, Hermens J, Seinen W and Vangestel K, 1994. Uptake of Hydrophobic halogenated aromatic-hydrocarbons from food by earthworms (*Eisenia andrei*). Archives of Environmental Contamination and Toxicology, 27, 260–265.
Belfroid A, Seinen W, Van Gestel K, Hermens J and VanLeeuwen K, 1995. Modelling the accumulation of hydrophobic organic chemicals in earthworms - Application of the equilibrium partitioning theory. Environmental Science and Pollution Research, 2, 5–15.
Bengtsson G, Hedlund K and Rundgren S, 1991. Selective odor perception in the soil collembola *Onychiurus armatus*. Journal of Chemical Ecology, 17, 2113–2125.
Bengtsson G, Hedlund K and Rundgren S, 1994. Food-dependent and density-dependent dispersal-evidence from a soil Collembolan. Journal of Animal Ecology, 63, 513–520.
Berke S, 2010. Functional Groups of Ecosystem Engineers: a Proposed Classification with Comments on Current Issues. Integrative and Comparative Biology, 50, 147–157.
Berruti A, Borriello R, Orgiazzi A, Barbera AC, Lumini E and Bianciotto V, 2014. Arbuscular mycorrhizal fungi and their value for ecosystem management. In: Grillo O (ed.). *Biodiversity – The Dynamic Balance of the Planet*. InTech, Rijeta, Croatia, pp. 159–191.
Berthet P, 1964. L'activite des oribatids d'une chenaie. Memoires de l'Institut Royale des Sciences Naturelles de Belgique, 152, 1–152.
Bertrand M, Barot S, Blouin M, Whalen J, de Oliveira T and Roger-Estrade J, 2015. Earthworm services for cropping systems. A review. Agronomy for Sustainable Development, 35, 553–567.
Beulke S, de Wilde T, Balderacchi M, Garreyn F, van Beinum V and Trevisan M, 2015. Scenario selection and scenario parameterisation for permanent crops and row crops on ridges in support of predicting environmental concentrations of plant protection products and their transformation products in soil. EFSA supporting publication 2015:EN-813, 170 pp. Available online: http://www.efsa.europa.eu/en/supporting/pub/813e
Beyer WN, 1996. Accumulation of chlorinated benzenes in earthworms. Bulletin of Environmental Contamination and Toxicology, 57, 729–736.
Bezchlebová J, Černohlávková J, Lána J, Sochová I, Kobetičová K and Hofman J, 2007a. Effects of toxaphene on soil organisms. Ecotoxicology and environmental safety, 68(3), 326–334.
Bezchlebová J, Černohlávková J, Kobetičová K, Lana J, Sochová I and Hofman J, 2007b. Effects of short-chain chlorinated paraffins on soil organisms. Ecotoxicology and environmental safety, 67, 206–211.

Bicho RC, Santos FCF, Gonçalves MFM, Soares AMVM and Amorim MJB, 2015. Enchytraeid reproduction testPLUS: hatching, growth and full life cycle test – an optional multi-endpoint test with *Enchytraeus crypticus*. Ecotoxicology, 24, 1053–1063.
Bidochka M, Kasperski J and Wild G, 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Canadian Journal Of Botany-Revue Canadienne De Botanique, 76, 1198–1204.
Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, Dai J, Dendooven L, Peres G, Tondoh JE, Cluzeau D and Brun JJ, 2013. A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science, 64, 161–182.
Bodelier PLE, 2011. Toward understanding, managing, and protecting microbial ecosystems. Frontiers in Microbiology, 2, 80.
Boekelheide K and Andersen ME, 2010. A mechanistic redefinition of adverse effects - a key step in the toxicity testing paradigm shift. ALTEX, 27, 243–252.
Boesten JJTI, 1986. Behaviour of herbicides in soil: simulation and experimental assessment. Doctoral Thesis, Institute for Pesticide Research, Wageningen, The Netherlands.
Boesten J, Koepp H, Adriaanse P, Brock T and Forbes V, 2007. Conceptual model for improving the link between exposure and effects in the aquatic risk assessment of pesticides. Ecotoxicology and Environmental Safety, 66, 291–308.
Bongers T, 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83, 14–19.
Bongers T and Bongers M, 1998. Functional diversity of nematodes. Applied Soil Ecology, 10, 239–251.
Bottinelli N, Jouquet P, Capowiez Y, Podwojewski P, Grimaldi M and Peng X, 2015. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil & Tillage Research, 146, 118–124.
Boyd WA and Williams PL, 2003. Comparison of the sensitivity of three nematode species to copper and their utility in aquatic and soil toxicity tests. Environmental toxicology and chemistry, 22(11), 2768–2774.
Brana F, Gonzalez-Quiros P, Prieto L and Gonzalez F, 2013. Spatial distribution and scale-dependent habitat selection by Eurasian Woodcocks *Scolopax rusticola* at the south-western limit of its continental breeding range in northern Spain. Acta Ornithologica, 48, 27–37.
Briones MJI, Carreira J and Ineson P, 1998. *Cognettia sphagnetorum* (Enchytraeidae) and nutrient cycling in organic soils: a microcosm experiment. Applied Soil Ecology, 9, 289–294.
Brock TCM, Alix A, Brown CD, Capri E, Gottesbüren BFF, Heimbach F, Lythgo CM, Schulz R and Streloke M (Eds) (2010). *Linking aquatic exposure and effects: risk assessment of pesticides*. SETAC Press & CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 398 pp.
Brock TCM, Hammers-Wirtz M, Hommen U, Preuss TG, Ratte HT, Roessink I, Strauss T and Van den Brink PJ, 2015. The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environmental Science and Pollution Research, 22, 1160–1174.
Bronick C and Lal R, 2005. Soil structure and management: a review. Geoderma, 124, 3–22.
Bross ID, 1985. Why proof of safety is much more difficult than proof of hazard. Biometrics, 41, 785–793.
Browde J, Pedigo L, Owen M, Tylka G and Levene B, 1994. Growth of soybean stressed by nematodes, herbicides and simulated insect defoliation. Agronomy Journal, 86, 968–974.
Brown SAE, Simpson AJ and Simpson MJ, 2009. 1H-NMR metabolomics of earthworm responses to sub-lethal PAH exposure. Environmental Chemistry, 6, 432–440.
Brühl C, Alscher A, Berger G, Bethwell C, Graef F, Hahn M, Schmidt T and Weber B, 2013. Protection of biodiversity in the risk assessment and risk management of pesticides with a focus on arthropods, soil organisms, and amphibians. Report of the Research and Development Project Nr 3709 65 421, 244 pp.
Brum PED and Araujo PB, 2007. The manca stages of *Porcellio dilatatus* Brandt (Crustacea, Isopoda, Oniscidea). Revista Brasileira de Zoologia, 24, 493–502.
Bruns E, Egeler P, Moser T, Römbke J, Scheffczyk A and Spörlein P, 2002. *Standardisierung und Validierung eines Bioakkumulationstests mit terrestrischen Oligochaeten*. Research project of the German Environment Agency (UBA), FKZ 298 64 416, ISSN 0722-186X, UBA Texte 07/02. UBA, Berlin.
Brussaard L and van Fassen H, 1994. Effects of compaction on soil biota and soil biological processes. In: Soane B, van Quwerkerk C (eds). *Soil Compaction in Crop Production*. Elsevier, Amsterdam, The Netherlands, pp. 215–235.
Brussaard L, de Ruiter PC and Brown GG, 2007a. Soil biodiversity for agricultural sustainability. Agriculture Ecosystems & Environment, 121, 233–244.
Brussaard L, Pulleman M, Ouédraogo E, Mando A and Six J, 2007b. Soil fauna and soil function in the fabric of the food web. Pedobiologia, 50, 447–462.
Burch J and Pearce T, 1990. Chapter 9: terrestrial gastropods. In: *Soil Biology Guide*. Ed Dindal D, John Wiley and Sons, New York, 1349 pp.
Burgers A and Raw F, 2012. *Soil Biology*. Academic Press, 532 pp.
Büscher B, Dressler W and Fletcher R, 2014. Nature™ Inc: Environmental conservation in the neoliberal age. Critical Green Engagements: Investigating the Green Economy and Its Alternatives, The University of Arizona Press, 298 pp.

Campbell CD, Chapman SJ, Cameron CM, Davidson MS and Potts JM, 2007. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substarte amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology, 69, 3593–3599.
Campiche S, L'Arnbert G, Tarradellas J and Becker-van Slooten K, 2007. Multigeneration effects of insect growth regulators on the springtail *Folsomia candida*. Ecotoxicology and Environmental Safety, 67, 180–189.
Campos-Herrera R, Gomez-Ros JM, Escuer M, Cuadra L, Barrios L and Gutierrez C, 2008. Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations from La Rioja (Northern Spain) under different agricultural management and their relationships with soil factors. Soil Biology & Biochemistry, 40, 1474–1484.
Capowiez Y, Bastardie F and Costagliola G, 2006. Sublethal effects of imidacloprid on the burrowing behaviour of two earthworm species: modifications of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. Soil Biology and Biochemistry, 38, 285–293.
Cardinale B, 2012. Biodiversity loss and its impact on humanity. Nature, 489, 59–67.
Caro G, Decaens T and Mathieu J, 2013a. The dispersal of the earthworm *Aporrectodea giardi* responds faster to habitat quality than to cumulative use of habitat in experimental conditions. Applied Soil Ecology, 71, 45–47.
Caro G, Decaens T, Lecarpentier C and Mathieu J, 2013b. Are dispersal behaviours of earthworms related to their functional group? Soil Biology & Biochemistry, 58, 181–187.
Caseiro I, Santos S, Sousa JP, Nogueira AJA and Soares AMVM, 2000. Optimization of culture conditions of *Porcellio dilatatus* (Crustacea: Isopoda) for laboratory test development. Ecotoxicology and Environmental Safety, 47, 285–291.
Chahartaghi M, Maraun M, Scheu S and Domes K, 2009. Resource depletion and colonization: a comparison between parthenogenetic and sexual Collembola species. Pedobiologia, 52, 181–189.
Chelinho S, Sautter KD, Cachada A, Abrantes I, Brown G, Duarte AC and Sousa JP, 2011. Carbofuran effects in soil nematode communities: using trait and taxonomic based approaches. Ecotoxicology and Environmental Safety, 74, 2002–2012.
Chelinho S, Domene X, Andrés P, Natal-da-Luz T, Norte C, Rufino C, Lopes I, Cachada A, Espíndola E, Ribeiro R, Duarte AC and Sousa JP, 2014. Soil microarthropod community testing: a new approach to increase the ecological relevance of effect data for pesticide risk assessment. Applied Soil Ecology, 83, 200–209.
Chibuike G, 2013. Use of mycorrhiza in soil remediation: a review. Scientific Research and Essays, 8, 1679–1687.
Choudhury A and Kennedy I, 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biology and Fertility of Soils, 39, 219–227.
Christl H, Bendall J, Bergtold M, Coulson M, Dinter A, Garlej B, Hammel K, Kabouw P, Sharples A, von Merey G, Vrbka S and Ernst G, 2015. Recalibration of the Earthworm Tier 1 Risk Assessment of Plant Protection Products. Integrated Environmental Assessment and Management, 12, 643–650.
Cockell CS, 2005. The value of microorganisms. Environmental Ethics, 27, 375–390.
Cockell CS and Jones HL, 2009. Advancing the case for microbial conservation. Oryx, 43, 520–526.
Coeurdassier M, Saint-Denis M, Gomot-de Vaufleury A, Ribera D and Badot P, 2001. The garden snail (*Helix aspersa*) as a bioindicator of organophosphorus exposure: effects of dimethoate on survival, growth, and acetylcholinesterase activity. Environmental Toxicology and Chemistry, 20, 1951–1957.
Coeurdassier M, Gomot-De Vaufleury A, Saint-Denis M, Ribera D, Narbonne JF and Badot PM, 2002. Effects of dimethoate on snail B-esterase and growth as a function of dose, time and exposure route in a laboratory bioassay. Biomarkers, 7, 138–150.
Cole L, Buckland S and Bardgett R, 2005. Relating microarthropod community structure and diversity to soil fertility manipulations in temperate grassland. Soil Biology & Biochemistry, 37, 1707–1717.
Cole JJ, Carpenter SR, Pace ML, Van de Bogert MC, Kitchell JL and Hodgson JR, 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecology Letters, 9, 558–568.
Comerford N, Franzluebbers A, Stromberger M, Morris L, Markewitz D and Moore R, 2013. Assessment and evaluation of soil ecosystem services. Soil Horizons, 54, 1–14.
Contreras-Ramos S, Alvarez-Bernal D and Dendooven L, 2006. *Eisenia foetida* increased removal of polycyclic aromatic hydrocarbons (PAHs) from soil. Environmental Pollution, 141, 396–401.
Contreras-Ramos S, Álvarez-Bernal D and Dendooven L, 2008. Removal of polycyclic aromatic hydrocarbons from soil amended with biosolid or vermicompost in the presence of earthworms (*Eisenia fetida*). Soil Biology and Biochemistry, 40, 1954–1959.
Cook J, Bruckart W, Coulson J, Goettel M, Humber R, Lumsden R, Maddox J, McManus M, Moore L, Meyer S, Quimby P, Stack J and Vaughn J, 1996. Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. Biological Control, 7, 333–351.
Cooke R, 2010. Conundrums with uncertainty factors (with discussion). Risk Analysis, 30, 330–338.
Cortet J, Gomot-De Vauflery A, Poinsot-Balaguer N, Gomot L, Texier C and Cluzeau D, 1999. The use of invertebrate soil fauna in monitoring pollutant effects. European Journal of Soil Biology, 35, 115–134.
Costa D, Bouchon D, Van Straalen NM, Sousa JP and Ribeiro R, 2013a. Copper tolerance and genetic diversity of *Porcellionides sexfasciatus* (Isopoda) at a highly contaminated mine habitat. Environmental Toxicology and Chemistry, 32, 884–888.

Costa D, Timmermans MJTN, Sousa JP, Ribeiro R, Roelofs D and Van Straalen NM, 2013b. Genetic structure of soil invertebrate populations: collembolans, earthworms and isopods. Applied Soil Ecology, 68, 61–66.
Cotrufo MF, Berg B and Kratz W, 1998. Increased atmospheric CO_2 and litter quality. Environmental Reviews, 6, 1–12.
Cragg R and Bardgett R, 2001. How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology & Biochemistry, 33, 2073–2081.
Crowther T, Boddy L and Jones T, 2012. Functional and ecological consequences of saprotrophic fungus-grazer interactions. Isme Journal, 6, 1992–2001.
Curl EA, Edwards PJ, Elliott C and Leahey JP, 1987. The conjugation and accumulation of metabolites of cypermethrin by earthworms. Pesticide Science, 20, 207–222.
Curl EA, Lartey R and Peterson CM, 1988. Interactions between root pathogens and soil microarthropods. Agriculture, Ecosystems & Environment, 24, 249–261.
Daam MA, Leitão S, Cerejeira MJ and Sousa JP, 2011. Comparing the sensitivity of soil invertebrates to pesticides with that of *Eisenia fetida*. Chemosphere, 85, 1040–1047.
Daily GC, Matson PA and Vitousek PM, 1997. Ecosystem services supplied by soil. In: Daily GC (ed.). *Nature's Services: societal Dependence on Natural Ecosystems*. Island Press, Washington DC. pp. 113–132.
Dalkvist T, Topping CJ and Forbes VE, 2009. Population-level impacts of pesticide-induced chronic effects on individuals depend more on ecology than toxicology. Ecotoxicology and Environmental Safety, 72, 1663–1672.
Dallinger R, Berger B, Triebskorn-Köhler R and Köhler H, 2001. Soil biology and ecotoxicology. In: Barker G (ed.). *The Biology of Terrestrial Molluscs*. CABI Publishing, Wallingford. pp. 489–525.
Dannowski M, 1994. Einfluss differenzierter mechanischer Bodenbelastung auf Bodengefügeeigenschaften, Durchwurzelbarkeit und Pflanzenertrag eines sandigen Moränenbodens. In: Bork H-R, Renger M, Alaily F, Roth C, Wessolek G (eds.). *Bodenökologie und Bodengenese*. Technische Universität, Berlin.
Darwin C, 1881. *The Formation of Vegetable Mould through the Action of Worms, with Observations on their Habits*. John Murray, London. 326 pp.
Das N, Kumar A and Singh P, 2015. Cyanobacteria, pesticides and rice interaction. Biodiversity and Conservation, 24, 995–1005.
Davison J, Öpik M, Zobel M, Vasar M, Metsis M and Moora M, 2012. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. PLoS ONE, 7, e41938.
De Jong FMW, Mensink BJWG, Smit CE and Montforts MHMM, 2005. Evaluation and use of ecotoxicological field studies for regulatory purposes of plant protection products in Europe. HERA, 11, 1157–1176.
De Jong F, van Beelen P, Smit C and Montforts M, 2006. Guidance for summarising earthworm field studies. RIVM report 601506006/2006. Bilthoven, The Netherlands. 47 pp.
De Jong FMW, Bakker FM, Brown K, Jilesen CJTJ, Posthuma-Doodeman CJAM, Smit CE, Van der Steen JJM and Van Eekelen GMA, 2010. Guidance for summarising and evaluating field studies with non-target arthropods. RIVM report 601712006/2010. 76 pp.
De Laender F, van den Brink PJ and Janssen CR, 2011. Functional redundancy and food-web functioning in linuron-exposed ecosystems. Environmental Pollution, 159, 3009–3017.
De Lange HJ, Sala S, Vighi M and Faber JH, 2010. Ecological vulnerability in risk assessment — a review and perspectives. Science of the Total Environment, 408, 3871–3879.
De Nardo E and Grewal P, 2003. Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with pesticides and plant growth regulators used in glasshouse plant production. Biocontrol Science and Technology, 13, 441–448.
De Ruiter PC, Neutel AM and Moore JC, 1998. Biodiversity in soil ecosystems: the role of energy flow and community stability. Applied Soil Ecology, 10, 217–228.
De Ruiter PC, Wolters V and Moore JC (eds), 2005a. *Dynamic Food Webs: multispecies Assemblages, Ecosystem Development, and Environmental Change*. Academic Press, San Diego, CA, 608 pp.
De Ruiter PC, Wolters V, Moore JC and Winemiller KO, 2005b. Food web ecology: playing Jenga and beyond. Science, 309, 68.
De Silva P and Amarasinghe N, 2008. Assessment of dimethoate toxicity on compost worm (*Eisenia andrei*) using earthworms avoidance test. Tropical Agricultural Research, 20, 25–33.
De Silva PMCS, Pathiratne A and van Gestel CAM, 2010. Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*. Applied Soil Ecology, 44, 56–60.
De Vaufleury A, 2015. Landsnail for ecotoxicological assessment of chemicals and soil contamination – ecotoxicological assessment of chemicals and contaminated soils using the terrestrial snail, *Helix aspersa*, at various stage of its life cycle: a review. In: Armon RH, Hänninen O (eds). *Environmental Indicators*. Springer, Dordrecht, The Netherlands. pp. 365–391.
De Vaufleury A, Coeurdassier M, Pandard P, Scheifler R, Lovy C, Crini N and Badot PM, 2006. How terrestrial snails can be used in risk assessment of soils. Environmental Toxicology and Chemistry, 25, 797–806.
Decaens T, 2010. Macroecological patterns in soil communities. Global Ecology and Biogeography, 19, 287–302.
Dechaine J, Ruan HH, Leon YSD and Zou XM, 2005. Correlation between earthworms and plant litter decomposition in a tropical wet forest of Puerto Rico. Pedobiologia, 49, 601–607.

Demon A and Eijsackers H, 1985. The effects of lindane and azinphosmethyl on survival time of soil animals, under extreme or fluctuating temperature and moisture conditions. Zeitschrift fur Angewandte Entomologie, 100, 504–510.
Detsis V, 2000. Vertical distribution of Collembola in deciduous forests under Mediterranean climatic conditions. Belgian Journal of Zoology, 130, 57–61.
Dewar A, Haylock L, May M, Beane J and Perry R, 2000. Glyphosate applied to genetically modified herbicide-tolerant sugar beet and 'volunteer' potatoes reduces populations of potato cyst nematodes and the number and size of daughter tubers. Annals of Applied Biology, 136, 179–187.
Diaz E, 2004. Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. International Microbiology, 7, 173–180.
Dickie A, 2007. Host preference, niches and fungal diversity. New Phytologist, 174, 230–233.
Didden W, 1990. Involvement of Enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. Biology and Fertility of Soils, 9, 152–158.
Diez-Ortiz M, Lahive E, Kille P, Powell K, Morgan A, Jurkschat K, Van Gestel C, Mosselmans J, Svendsen C and Spurgeon D, 2015. Uptake routes and toxicokinetics of silver nanoparticles and silver ions in the earthworm *Lumbricus rubellus*. Environmental Toxicology and Chemistry, 34, 2263–2270.
Dijksterhuis J, van Doorn T, Samson R and Postma J, 2011. Effects of Seven Fungicides on Non-Target Aquatic Fungi. Water Air and Soil Pollution, 222, 421–425.
Dinter A, Oberwalder C, Kabouw P, Coulson M, Ernst G, Leicher T, Miles M, Weyman G and Klein O, 2013. Occurrence and distribution of earthworms in agricultural landscapes across Europe with regard to testing for responses to plant protection products. Journal of soils and sediments, 13, 278–293.
Dittbrenner N, Capowiez Y, Köhler HR and Triebskorn R, 2012. Stress protein response (Hsp70) and avoidance behaviour in *Eisenia fetida*, *Aporrectodea caliginosa* and *Lumbricus terrestris* when exposed to imidacloprid. Journal of Soils and Sediments, 12, 198–206.
Dittmer S and Schrader S, 2000. Longterm effects of soil compaction and tillage on Collembola and straw decomposition in arable soil. Pedobiologia, 44, 527–538.
Dixon PM and Pechmann JHK, 2005. A statistical test to show negligible trend. Ecology, 86, 1751–1756.
Dominati E, Patterson M and Mackay A, 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecological Economics, 69, 1858–1868.
Dong L and Zhang K, 2006. Microbial control of plant-parasitic nematodes: a five-party interaction. Plant and Soil, 288, 31–45.
Dorato MA and Engelhardt JA, 2005. The no-observed-adverse-effect-level in drug safety evaluations: Use, issues, and definition(s). Regulatory Toxicology and Pharmacology, 42, 265–274.
Drobne D, Blaz M, Van Gestel CAM, Leser V, Zidar P, Jemec A and Trebse P, 2008. Toxicity of imidacloprid to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea). Chemosphere, 71, 1326–1334.
Dromph KM, 2003. Effect of starvation on phototaxis and geotaxis of collembolans. European Journal of Soil Biology, 39, 9–12.
Druart C, Scheifler R and de Vaufleury A, 2010. Towards the development of an embryotoxicity bioassay with terrestrial snails: Screening approach for cadmium and pesticides. Journal of Hazardous Materials, 184, 26–33.
Druart C, Millet M, Scheifler R, Delhomme O, Raeppel C and de Vaufleury A, 2011. Snails as indicators of pesticide drift, deposit, transfer and effects in the vineyard. Science of the Total Environment, 409, 4280–4288.
Druart C, Scheifler R, Millet M and de Vaufleury A, 2012. Landsnail eggs bioassays: a new tool to assess embryotoxicity of contaminants in the solid, liquid or gaseous phase of soil. Applied Soil Ecology, 53, 56–64.
Ducklow H, 2008. Microbial services: challenges for microbial ecologists in a changing world. Aquatic Microbial Ecology, 53, 13–19.
Duffield S and Aebischer N, 1994. The effect of spatial scale of treatment with dimethoate on invertebrate population recovery in winter wheat. Journal of Applied Ecology, 31, 263–281.
Duffus JH, Nordberg M and Templeton DM, 2007. IUPAC GLOSSARY of terms used in toxicology-, 2nd edition. International Union of Pure and Applied Chemistry/Chemistry and Human Health Division. Pure and Applied Chemistry, 79, 1153–1344.
Duffy JE, 2002. Biodiversity and ecosystem function: the consumer connection. Oikos, 99, 201–219.
Duncan J, Hinchcliffe A and Palmquist K, 2009. Evidence of potential long term effects in (aquatic and terrestrial) invertebrates after short term pulsed exposure. Literature reviews on ecotoxicology of chemicals with special focus on plant protection products. EFSA supporting publication 2009: CFT/EFSA/PPR/2008/01, 87 pp.
Duncan L, Stuart R, El-Borai F, Campos-Herrera R, Pathak E, Giurcanu M and Graham JH, 2013. Modifying orchard planting sites conserves entomopathogenic nematodes, reduces weevil herbivory and increases citrus tree growth, survival and fruit yield. Biological Control, 64, 26–36.
Dupont L, Gresille Y, Richard B, Decaens T and Mathieu J, 2015. Dispersal constraints and fine-scale spatial genetic structure in two earthworm species. Biological Journal of the Linnean Society, 114, 335–347.
European Commission (EC), 2000. Guidance Document on persistence in soil (SANCO/9188VI/1997) revision 8 of 12 July 2000.
European Commission (EC), 2002. Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) revision 2, final. 1–39.

European Commission (EC), 2016. Draft authorisation of Plant Protection Products for Seed Treatment (draft), SANCO/10553/2012, January 2014.
ECHA (European Chemical Agency), 2014. Principles for environmental risk assessment of the sediment compartment. Proceedings of the Topical Scientific Workshop, Helsinki, 7–8 May 2013, 82 pp.
ECHA (European Chemical Agency), 2016. Topical scientific workshop on soil risk assessment. Workshop Proceedings. Helsinki, 7–8 October, 61 pp.
Edwards CA, 2004. *Earthworm Ecology*. 2nd Edition. CRC Press, Washington DC. 456 pp.
Edwards CA and Bohlen PJ, 1996. *The Biology and Ecology of Earthworms*. 3rd Edition. Chapman & Hall, London 426 pp.
Edwards PJ and Coulson JM, 1992. Choice of earthworm species for laboratory tests. In: Greig-Smith PW, Becker H, Edwards PJ, Heimbach F (eds.). *Ecotoxicology of Earthworms*. Intercept, Andover, pp. 36–43.
EFSA (European Food Safety Authority), 2005. Scientific Opinion of the Panel on Plant Health, Plant Protection Products and their Residues on a request from EFSA on the appropriateness of using the current FOCUS surface water scenarios for estimating exposure for risk assessment in aquatic ecotoxicology in the context of Council Directive 91/414/EEC. EFSA Journal 2005;3(1):145, 31 pp. doi:10.2903/j.efsa.2005.145
EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues (PPR) on a request from EFSA related to the assessment of the acute and chronic risk to aquatic organisms with regard to the possibility of lowering the uncertainty factor if additional species were tested. EFSA Journal 2006;4(1):301, 45 pp. doi:10.2903/j.efsa.2006.301
EFSA (European Food Safety Authority), 2007. Scientific opinion of the panel on plant protection products and their residues on the final report of the FOCUS Air Working Group on Pesticides in Air: consideration for exposure assessment. (SANCO/10553/2006 draft 1 (13 July 2006). EFSA Journal 2007;5(7):513, 30 pp. doi:10.2903/j.efsa.2007.513
EFSA (European Food Safety Authority), 2009a. Guidance Document on Risk Assessment for birds and mammals on request from EFSA. EFSA Journal 2009;7(12):1438. doi:10.2903/j.efsa.2009.1438
EFSA (European Food Safety Authority), 2009b. Outcome of the public consultation on the existing Guidance Documents on Aquatic and Terrestrial Ecotoxicology under Directive 91/414/EC1. EFSA Journal 2009;7(11):1375. doi:10.2903/j.efsa.2009.1375
EFSA (European Food Safety Authority), 2009c. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR). The usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects. EFSA Journal 2009;7(1):922, 90 pp. doi:10.2903/j.efsa.2009.922
EFSA (European Food Safety Authority), 2010. Scientific Report of EFSA: Selection of Scenarios for Exposure of Soil Organisms to Plant Protection Products. In support of Revision of the Guidance Document on Persistence in Soil under Council Directive 91/414/EEC and Council Regulation 11/07/2009 (SANCO/9188/VI/97 rev. 8, 12.07.2000. EFSA Journal 2010;8(6):1642, 82 pp. doi:10.2903/j.efsa.2010.1642
EFSA (European Food Safety Authority), 2013. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295, 82 pp. doi:10.2903/j.efsa.2013.3295
EFSA (European Food Safety Authority), 2014a. EFSA's 19th Scientific Colloquium on Biodiversity as protection goal in environmental risk assessment for EU agro-ecosystems. EFSA Supporting Publication, doi:10.2903/sp.efsa.2014.EN-583
EFSA (European Food Safety Authority), 2014b. Guidance on Expert Knowledge Elicitation in Food and 2620 Feed Safety Risk Assessment. EFSA Journal 2014;12(6):3734. doi:10.2903/j.efsa.2014.3734
EFSA (European Food Safety Authority), 2016. Draft Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. Available online: https://www.efsa.europa.eu/sites/default/files/consultation/160715.pdf
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010a. Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal 2010;8(10):1821, 1855 pp. doi:10.2903/j.efsa.2010.1821
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010b. Scientific Opinion on outline proposals for assessment of exposure of organisms to substances in soil. EFSA Journal 2010;8(1):1442, 1438 pp. doi:10.2903/j.efsa.2010.1442
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010c. Scientific Opinion on the importance of the soil litter layer in agricultural areas. EFSA Journal 2010;8(6):1625, 21 pp. doi:10.2903/j.efsa.2010.1625
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010d. Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. EFSA Journal 2010;8(10):1820, 1877 pp. doi:10.2903/j.efsa.2010.1820

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2012. Scientific Opinion on the science behind the guidance for scenario selection and scenario parameterisation for predicting environmental concentrations of plant protection products in soil. EFSA Journal 2012;10(2):2562, 30 pp. doi:10.2903/j.efsa.2012.2562

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(6):3268. doi:10.2903/j.efsa.2013.3268

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2014a. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non target terrestrial plants. EFSA Journal 2014;12(7):3800, 3163 pp. doi:10.2903/j.efsa.2014.3800

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2014b. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA Journal 2014;12(3):3589, 92 pp. doi:10.2903/j.efsa.2014.3589

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015a. Scientific opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996, 3212 pp. doi:10.2903/j.efsa.2015.3996

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015b. Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. EFSA Journal 2015;13(7):476, 4145 pp. doi:10.2903/j.efsa.2015.4176

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015c. Statement on the FERA guidance proposal: 'Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments' (FERA, 2012). EFSA Journal 2015;13(7):4175, 4154 pp. doi:10.2903/j.efsa.2015.4175

EFSA Scientific Committee (SC), 2015. Guidance on Uncertainty in EFSA Scientific Assessment. Available online: http://www.efsa.europa.eu/sites/default/files/consultation/150618.pdf

EFSA Scientific Committee (SC), 2016a. Recovery in environmental risk assessments at EFSA. EFSA Journal 2016;14(2):4313 doi:10.2903/j.efsa.2016.4313

EFSA Scientific Committee (SC), 2016b. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. doi:10.2903/j.efsa.2016.4499

EFSA Scientific Committee (SC), 2016c. Coverage of endangered species in environmental risk assessments at EFSA. EFSA Journal 2016;14(6):4312, 124 pp. doi:10.2903/j.efsa.2016.4312

Egerer S, Kotschik P, Felsmann D, König W and Pieper S, 2015. Which is the relevant soil depth to assess the risk for soil organisms exposed to plant protection products? Proceedings of the 25th SETAC Europe Annual Meeting, Barcelona, Spain.

Eijsackers H, 2011. Earthworms as colonizers of natural and cultivated soil environments. Applied Soil Ecology, 50, 1–13.

Eijsackers H, Van Gestel C, De Jonge S, Muijis B and Slijkerman D, 2001. PAH-Polluted Dredged Peat Sediments and Earthworms: a Mutual Inference. Journal of Ecotoxicology, 10, 35–50.

Eisenlord S, Zak D and Upchurch R, 2012. Dispersal limitation and the assembly of soil Actinobacteria communities in a long-term chronosequence. Ecology and Evolution, 2, 538–549.

Emmerling C and Strunk H, 2012. Active dispersal of the endo-anecic earthworm *Aporrectodea longa* (Ude) in an experimental box. Soil Organisms, 84, 491–498.

Engenheiro EL, Hankard PK, Sousa JP, Lemos M, Weeks JM and Soares AMVM, 2005. Influence of dimethoate on acetylcholinesterase activity and locomotor function in terrestrial isopods. Environmental Toxicology and Chemistry, 24, 603–609.

EPPO (European and Mediterranean Plant Protection Organization), 2003. Environmental risk assessment scheme for plant protection products. Chapter 8: soil organisms and functions. EPPO Bulletin, 33, 195–209.

Ernst G, Kabouw P, Barth M, Marx MT, Frommholz U, Royer S and Friedrich S, 2016. Assessing the potential for intrinsic recovery in a Collembola two-generation study: possible implementation in a tiered soil risk assessment approach for plant protection products. Ecotoxicology, 25, 1–14.

Eskils K and Lövgren A, 2011. Distribution of *Bacillus thuringiensis* subsp. *israelensis* in soil of a Swiss wetland reserve after 22 years of mosquito control. Applied and Environmental Microbiology, 77, 3663–3668.

Evans PG, 2003. Study report no. RJ3219B. Company confidential.

Fang H, Cai L, Yang Y, Ju F, Li X, Yu Y and Zhang T, 2014. Metagenomic analysis reveals potential biodegradation pathways of persistent pesticides in freshwater and marine sediments. Science of the Total Environment, 470–471, 983–992.

Feld L, Hjelmsø MH, Nielsen MS, Jacobsen AD, Rønn R, Ekelund F, Krogh PH, Strobel BW and Jacobsen CS, 2015. Pesticide Side Effects in an Agricultural Soil Ecosystem as Measured by amoA Expression Quantification and Bacterial Diversity Changes. PLoS ONE, 10, 1–20.

Fent G, Löffler D and Kubiak R. 1999. Ermittlung der Eindringtiefe und Konzentrationsverteilung gesprühter Pflanzenschutzmittelwirkstoffe in den Boden zur Berechnung der PEC-Boden. German Federal Environment Agency Umweltbundesamt FKZ 360 03 018, final report UBA, Berlin.

Ferreira NGC, Morgado R, Santos MJG, Soares AMVM and Loureiro S, 2015. Biomarkers and energy reserves in the isopod *Porcellionides pruinosus*: the effects of long-term exposure to dimethoate. Science of the Total Environment, 502, 91–102.
Ferrero A and Lipiec J, 2000. Determining the effect of trampling on soils in hillslope-woodlands. International Agrophysics, 14, 9–16.
Ferris H and Bongers T, 2009. Indices developed specifically for analysis of nematode assemblages. In: Wilson MJ and Kakouli-Duarte T (eds). *Nematodes as Environmental Indicators*. CAB International, Wallingford. pp. 124–145.
Ferris H and Tuomisto H, 2015. Unearthing the role of biological diversity in soil health. Soil Biology and Biochemistry, 85, 101–109.
Ferris H, Bongers T and de Goede R, 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology, 18, 13–29.
Fierer N, 2008. Microbial biogeography: patterns in microbial diversity across space and time. In: Zengler K (ed.). Accessing Uncultivated Microorganisms: from the Environment to Organisms and Genomes and Back. ASM Press, Washington, DC. pp. 95–115.
Fierer N, Barberan A and Laughlin DC, 2014. Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. Frontiers in Microbiology, 5, 614.
Filser J, 2002. The role of Collembola in carbon and nitrogen cycling in soil. Pedobiologia, 46, 234–245.
Finlay B, 2002. Global dispersal of free-living microbial eukaryote species. Science, 296, 1061–1063.
Fiscus DA and Neher DA, 2002. Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances. Ecological Applications, 12, 565–575.
Fitter AH, Gilligan CA, Hollingworth K, Kleczkowski A, Twyman RM, Pitchford JW and Programme NSB, 2005. Biodiversity and ecosystem function in soil. Functional Ecology, 19, 369–377.
Flowers M and Lal R, 1998. Axle load and tillage effects on soil physical properties and soybean grain yield on a mollic ochraqualf in northwest Ohio. Soil & Tillage Research, 48, 21–35.
Focks A, ter Horst M, ven den Berg E, Baveco H and van denBrink PJ, 2014. Integrating chemical fate and population-level effect models for pesticides at landscape scale: New options for risk assessment. Ecological Modelling, 280, 102–116.
FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use), 1997. Soil persistence models and EU registration. Report of the FOCUS Soil Modelling Workgroup.
FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use), 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001 rev. 2, 245 pp.
FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use), 2007. Final Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment. SANCO/10422/2005, version 2.0, rev. 2.
FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use), 2008. Pesticides in air: considerations for exposure assessment. Report of the FOCUS Working Group on Pesticides in Air. EC Document Reference SANCO/10553/2006 rev. 2.
Fontanetti CS, Nogarol LR, de Souza RB, Perez DG and Maziviero GT, 2011. Bioindicators and biomarkers in the assessment of soil toxicity. Chapter 8. In: Pascucci S (ed.). *Soil Contamination*. InTech, Rijeka. 143–168.
Forbes VE and Calow P, 2002. Population growth rate as a basis for ecological risk assessment of toxic chemicals. *Philosophical transactions of the Royal Society of London*. Series B, Biological sciences. 357, 1299–1306.
Förster B, Van Gestel CAM, Koolhaas JE, Nentwig G, Rodrigues JML, Sousa JP, Jones SE and Knacker T, 2004. Ring-testing and field-validation of a terrestrial model ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on organic matter breakdown and soil fauna feeding activity. Ecotoxicology, 13, 129–141.
Fountain M and Hopkin S, 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod. Annual Review of Entomology, 50, 201–222.
Frampton GK, Jaensch S, Scott-Fordsmand JJ, Römbke J and Van den Brink PJ, 2006. Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. Environmental Toxicology and Chemistry, 25, 2480–2489.
Freude H, Harde K-W, Lohse GA, Klausnitzer B and Müller-Motzfeld G, 2005. *Die Käfer Mitteleuropas Band 2, Adephaga 1 Carabidae (Laufkäfer)*. Elsevier Spektrum Akademischer Verlag, Heidelberg. 521 pp.
Friend J and Chan K, 1995. Influence of cropping on the population of a native earthworm and consequent effects on hydraulic properties of vertisols. Australian Journal of Soil Research, 33, 995–1006.
Frouz J, Elhottova D, Kuraz V and Sourkova M, 2006. Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: results of a field microcosm experiment. Applied Soil Ecology, 33, 308–320.
Garcia del Pino F and Jove M, 2005. Compatibility of entomopathogenic nematodes with fipronil. Journal of Helminthology, 79, 333–337.
Garcia del Pino F and Morton A, 2010. Synergistic effect of the herbicides glyphosate and MCPA on survival of entomopathogenic nematodes. Biocontrol Science and Technology, 20, 483–488.

Garcia M, Römbke J, de Brito MT and Scheffczyk A, 2008. Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. Environmental Pollution, 153, 450–456.
Garcia-Palacios P, Maestre F, Kattge J and Wall D, 2013. Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecology Letters, 16, 1418.
Gardner R, Engelhardt K, Elmore A and Cadol D, 2014. A traits-based model of species diversity. Ecological Modelling, 288, 178–194.
Garthwaite D, Sinclair C, Glass R, Pote A, Trevisan M, Sacchettini G, Spanoghe P, Ngoc KD, Fevery D, Machera K, Charistou A, Nikolopoulou D, Arapaki N, Tsakirakis A, Gerritsen-Ebben R, Spaan S, González FE, Stobiecki S, Śliwiński W, Stobiecki T and Hakaite P, 2015. Collection of pesticide application data in view of performing Environmental Risk Assessments for pesticides. EFSA Supporting publication, 2015:EN-846, 246 pp. http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2015.EN-846/pdf
Gascon C, Brooks TM, Contreras-MacBeath T, Heard N, Konstant W, Lamoreux J, Launay F, Maunder M, Mittermeier RA, Molur S, Al Mubarak RK, Parr MJ, Rhodin AGJ, Rylands AB, Soorae P, Sanderson JG and Vie J-C, 2015. The importance and benefits of species. Current Biology, 25, R431–R438.
Gayle G, Raczkowski C and Mims M, 2005. Evaluating soil physical properties at a high elevation eco-tourism park in Jamaica. Proceedings of the American Society of Agricultural and Biological Engineers, St. Joseph, Michigan.
Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A and Rubin DB, 2013. *Bayesian Data Analysis*. 3rd Edition. Chapman and Hall/CRC Press, London, UK.
Georgis R, Kaya HK and Gaugler R, 1991. Effect of Steinernematid and Heterorhabditid nematodes (Rhabditida, Steinernematidae, and Heterorhabditidae) on non-target arthropods. Environmental Entomology, 20, 815–822.
Gevao B, Mordaunt C, Semple K, Piearce T and Jones K, 2001. Bioavailability of nonextractable (bound) pesticide residues to earthworms. Environment Science Technology, 35, 501–507.
Gianinazzi S, Gollotte A, Binet M, van Tuinen D, Redecker D and Wipf D, 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza, 20, 519–530.
Giesy JP and Graney R, 1989. Recent developments in and intercomparisons of acute and chronic bioassays and bioindicators. Hydrobiologia, 188(189), 21–60.
Gilreath J and Santos B, 2004. Herbicide dose and incorporation depth in combination with 1,3-dichloropropene plus chloropicrin for Cyperus rotundus control in tomato and pepper. Crop Protection, 23, 205–210.
Giovannetti M, Turrini A, Strani P, Sbrana C, Avio L and Pietrangeli B, 2006. Mycorrhizal fungi in ecotoxicological studies: soil impact of fungicides, insecticides and herbicides. Prevention Today, 2, 47–62.
Gitay H, Wilson JB and Lee WG, 1996. Species redundancy: a redundant concept? Journal of Ecology, 84, 121–124.
Gobas F, Burkhard L, Doucette W, Sappington K, Verbruggen E, Hope B, Bonnell M, Arnot J and Tarazona J, 2015. Review of existing terrestrial bioaccumulation models and terrestrial bioaccumulation modeling needs for organic chemicals. Integrated Environmental Assessment and Management, 12, 123–134.
Gobster P, Nassauer J, Daniel T and Fry G, 2007. The shared landscape: what does aesthetics have to do with ecology? Landscape Ecology, 22, 959–972.
Gosteli M, 1996. Diversities of snail faunas and ecological relationships between snail community and vegetation in dry habitats of the northern Swiss Jura (Gastropoda: Prosobranchia et Pulmonata). Malakol. Abh. Staatl. Mus. Tierkd. Dresden, 18, 107–120.
Goszczynski J, Jedrzejewska B and Jedrzejewski W, 2000. Diet composition of badgers (Meles meles) in a pristine forest and rural habitats of Poland compared to other European populations. Journal of Zoology, 250, 495–505.
Graf F and Frei M, 2013. Soil aggregate stability related to soil density, root length, and mycorrhiza using site-specific *Alnus incana* and *Melanogaster variegatus* s.l. Ecological Engineering, 57, 314–323.
Graham P, 2008. *Ecology of the root-nodule bacteria of legumes*. In: Nitrogen-fixing legume symbioses. Eds Dilworth MJ, James EK, Sprent JI, Newton WE (eds). Dordrecht, the Netherlands: Springer, 23–58.
Graveland J and van der Wal R, 1996. Decline in snail abundance due to soil acidification causes eggshell defects in forest passerines. Oecologia, 105, 351–360.
Graveland J and Van Gijzen T, 1994. Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. Ardea, 82, 299–314.
Green S, Inbar E, Michel F, Hadar Y and Minz D, 2006. Succession of bacterial communities during early plant development: Transition from seed to root and effect of compost amendment. Applied and Environmental Microbiology, 72, 3975–3983.
Greig-Smith PW, Backer H, Edwards PJ and Heimbach F, 1992. Ecotoxicology of earthworms. Eds Greig-Smith PW, Backer H, Edwards PJ, Heimbach F. Intercept Ltd, Andover, Hants, UK, 269 pp.
Grewal PS, 2002. Formulation and application technology. In: Nematology Entomopathogenic (ed). *Gaugler R*. CABI publishing, Wallingford, UK. pp. 265–288.
Griffiths BS, Wood S and Cheshire MV, 1989. Mineralization of C-14-labelled plant material by Porcelio Scaber (Crustacea, Isopoda). Pedobiologia, 33, 355–360.
Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, Ekelund F, Sorensen SJ, Muller S and Bloem J, 2001. An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biology & Biochemistry, 33, 1713–1722.

Guggenberger G, 2005. Humification and mineralization in soils. Microorganisms in Soils: Roles in Genesis and Functions, 3, 85–106.
Guidi V, Patocchi N, Lüthy P and Tonolla M, 2011. Distribution of Bacillus thuringiensis subsp. israelensis in Soil of a Swiss Wetland Reserve after 22 Years of Mosquito Control. Applied and environmental microbiology, 77, 3663–3668.
Gyldenkaerne S and Jorgensen S, 2000. Modelling the bioavailability of pesticides to soil-dwelling organisms. Ecological Modelling, 132, 203–230.
Haegerbaeumer A, Höss S, Ristau K, Claus E, Möhlenkamp C, Heininger P and Traunspurger W, 2016. A comparative approach using ecotoxicological methods from single-species bioassays to model ecosystems. Environmental Toxicology and Chemistry, 9999, 1–11.
Hallin S, Jones CM, Schloter M and Philippot L, 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. Isme Journal, 3, 597–605.
Hamza M and Anderson W, 2005. Soil compaction in cropping systems - A review of the nature, causes and possible solutions. Soil & Tillage Research, 82, 121–145.
Hanlon R and Anderson J, 1980. Influence of macroarthropod feeding activities on microflora in decomposing oak leaves. Soil Biology & Biochemistry, 12, 255–261.
Harrier L and Watson C, 2004. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Management Science, 60, 149–157.
Hartnik T, Sverdrup L and Jensen J, 2008. Toxicity of the pesticide alpha-cypermethrin to four soil nontarget invertebrates and implications for risk assessment. Environmental Toxicology and Chemistry, 27, 1408–1415.
Hasegawa M and Takeda H, 1995. Changes in feeding attributes of 4 Collembolan populations during the decomposition process of pine needles. Pedobiologia, 39, 155–169.
Hassall S, Visser D and Parkinson A, 1986. Vertical migration of *Onychiurus subtenuis* Collembola in relation to rainfall and microbial activity. Pedobiologia, 29, 175–182.
Hassall M, Turner JG and Rands MRW, 1987. Effects of terrestrial Isopods on the decomposition of woodland leaf litter. Oecologia, 72, 597–604.
He ZL, Deng Y and Zhou JZ, 2012. Development of functional gene microarrays for microbial community analysis. Current Opinion in Biotechnology, 23, 49–55.
Heemsbergen D, Berg M, Loreau M, van Haj J, Faber J and Verhoef H, 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science, 306, 1019–1020.
Heimbach F, 1997. Comparison of the sensitivities of an earthworm (*Eisenia fetida*) reproduction test and a standarized field test on grassland. In: Sheppard SC, Bembridge JD, Holmstrup M and Posthuma L (eds). *Advances in earthworm ecotoxicology*. SETAC.
Heiniger C, Barot S, Ponge J, Salmon S, Botton-Divet L, Carmignac D and Dubs F, 2014. Effect of habitat spatiotemporal structure on collembolan diversity. Pedobiologia, 57, 103–117.
Heisler C and Kaiser E, 1995. Influence of agricultural traffic and crop management on Collembola and microbial biomass in arable soil. Biology and Fertility of Soils, 19, 159–165.
Henderson CF and Tilton EW, 1955. Tests with acaricides against the brow wheat mite. Journal of Economic Entomologu, 48, 157–161.
Heneghan L and Bolger T, 1998. Soil microarthopod contribution to forest ecosystem processes: the importance of observational scale. Plant and Soil, 205, 113–124.
Hickman Z and Reid B, 2008. Earthworm assisted bioremediation of organic contaminants. Environment International, 34, 1072–1081.
Hodge S, Webster KM, Booth L, Hepplethwaite V and O'Halloran K, 2000. Non-avoidance of organophosphate insecticides by the earthworm *Aporrectodea caliginosa* (Lumbricidae). Soil Biology & Biochemistry, 32, 425–428.
Hoekstra JA and van Ewijk PH, 1993. Alternatives for the no-observed-effect level. Environmental Toxicology and Chemistry, 12, 187–194.
Hof AR and Bright PW, 2010. The impact of grassy field margins on macro-invertebrate abundance in adjacent arable fields. Agriculture Ecosystems & Environment, 139, 280–283.
Hole D, Perkins A, Wilson J, Alexander I, Grice P and Evans ABC, 2005. Does organic farming benefit biodiversity? Biological Conservation, 122, 113–130.
Hoodless AN and Hirons GJM, 2007. Habitat selection and foraging behaviour of breeding Eurasian Woodcock Scolopax rusticola: a comparison between contrasting landscapes. Ibis, 149, 234–249.
Hopkin SP, 1997. *Biology of the Springtails (Insecta: Collembola)*. Ed Hopkin SP. Oxford University Press, New York, USA. 330 pp.
Hopkins DW and Dungait JAJ, 2010. Soil Microbiology and Nutrient Cycling. In: *Soil Microbiology and Sustainable Crop Production*. Eds Dixon GR and Tilston EL. Springer, 59–80.
Horn R, Taubner H, Wuttke M and Baumgartl T, 1994. Soil physical properties related to soil structure. Soil & Tillage Research, 30, 187–216.
Horton T and Bruns T, 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology, 10, 1855–1871.

Höss S, Jänsch S, Moser T, Junker T and Römbke J, 2009. Assessing the toxicity of contaminated soils using the nematode *Caenorhabditis elegans* as test organism. Ecotoxicology and Environmental Safety, 72, 1811–1818.
Höss S, Reiff N, Nguyen HT, Jehle JA, Hermes H and Traunspurger W, 2014. Small-scale microcosms to detect chemical induced changes in soil nematode communi-ties – Effects of crystal proteins and Bt-maize plant material. Science of the Total Environment, 472, 662–671.
Høye TT, Skov F and Topping CJ, 2012. Evaluating wildlife responses to land-use scenarios with agent-based models. Ecological Indicators, 20, 221–227.
Hu CW, Zhang LJ, Wang WL, Cui YB and Li M, 2014. Evaluation of the combined toxicity of multi-walled carbon nanotubes and sodium pentachlorophenate on the earthworm *Eisenia fetida* using avoidance bioassay and comet assay. Soil Biology & Biochemistry, 70, 123–130.
Huguier P, Manier N, Owojori OJ, Bauda P, Pandard P and Roembke J, 2015. The use of soil mites in ecotoxicology: a review. Ecotoxicology, 24, 1–18.
Huhta V, Persson T and Setala H, 1998. Functional implications of soil fauna diversity in boreal forests. Applied Soil Ecology, 10, 277–288.
Hunt H and Wall D, 2002. Modelling the effects of loss of soil biodiversity on ecosystem function. Global Change Biology, 8, 33–50.
Hussain S, Siddique T, Saleem M, Arshad M and Khalid A, 2009. Chapter 5: Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Advances in Agronomy, 102, 159–200.
Iglesias J, Castillejo J and Castro R, 2003. The effects of repeated applications of the molluscicide metaldehyde and the biocontrol nematode *Phasmarhabditis hermaphrodita* on molluscs, earthworms, nematodes, acarids and collembolans: a two-year study in north-west Spain. Pest Management Science, 59, 1217–1224.
Imfeld G and Vuilleumier S, 2012. Measuring the effects of pesticides on bacterial communities in soil: a critical review. European Journal of Soil Biology, 49, 22–30.
Inglis G, Goettel M, Butt T and Strasser H, 2001. Use of hyphomycetous fungi for managing insect pests. *Fungi as Biocontrol Agents: progress, Problems and Potential*. Eds Butt TM, Jackson C, Magan N. CAB International, Wallingford, UK, pp. 23–69.
ISO (International Organisation for Standardization), 1999. Soil quality: effects of pollutants on earthworms. Part 3: guidance on the determination of effects in field situations (ISO 11268-3). Revised in 2014. 1–8.
ISO (International Organisation for Standardization), 2006. Soil quality — effects of pollutants on juvenile land snails (Helicidae) — determination of the effects on growth by soil contamination (ISO 15952).
ISO (International Organisation for Standardization), 2008. Soil quality – Avoidance test for determining the quality of soils and effects of chemicals on behaviour – Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*) (ISO 17512-1).
ISO (International Organisation for Standardization), 2009. Soil quality — effects of pollutants on mycorrhizal fungi — spore germination test (ISO/TS 10832).
ISO (International Organisation for Standardization), 2011. Soil quality – avoidance test for determining the quality of soils and effects of chemicals on behaviour – Part 2: test with collembolans (*Folsomia candida*) (ISO 17512-2).
ISO (International Organisation for Standardization), 2014. Soil Quality: effects of pollutants on earthworms. Part 3: guidance on the determination of effects in field situations (ISO 11268-3).
Jager R, Fleuren E and Hogendoorn GdK, 2003. Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). Environmental Science and Technology, 37, 3399–3404.
Jänsch S, Garcia M and Römbke J, 2005. Acute and chronic isopod testing using tropical *Porcellionides pruinosus* and three model pesticides. European Journal of Soil Biology, 41, 143–152.
Jarratt N and Thompson N, 2009. Comparison between the sensitivity of enchytraeids and lumbricidae to chemicals, in particular plant protection products. EFSA Supporting publication 2009: CFT/EFSA/PPR/2008/01, 196 pp.
Jegou D, Schrader S, Diestel H and Cluzeau D, 2001. Morphological, physical and biochemical characteristics of burrow walls formed by earthworms. Applied Soil Ecology, 17, 165–174.
Jennings T and Barkham J, 1976. Quantitative study of feeding in woodland by the slug Anon ater. Oikos, 27, 168–173.
Jimenez JJ, Cepeda A, Decaens T, Oberson A and Friesen DK, 2003. Phosphorus fractions and dynamics in surface earthworm casts under native and improved grasslands in a Colombian savanna Oxisol. Soil Biology & Biochemistry, 35, 715–727.
Johnson DR, Czechowska K, Chevre N and van der Meer JR, 2009. Toxicity of triclosan, penconazole and metalaxyl on Caulobacter crescentus and a freshwater microbial community as assessed by flow cytometry. Environmental Microbiology, 11, 1682–1691.
Jones CG, Lawton JH, Shachak M, Samson FB and Knopf FL, 1996. reprinted Organisms as ecosystem engineers. Oikos, 69, 373–386.
Jones M, Durall D and Tinker P, 1998. Comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production. New Phytologist, 140, 125–134.
Jones C, Gutierrez J, Groffman P and Shachak M, 2006. Linking ecosystem engineers to soil processes: a framework using the Jenny State Factor Equation. European Journal of Soil Biology, 42, S39–S53.

Jones A, Panagos P, Barcelo S, Bouraoui C, Bosco C, Dewitte O, Gardi C, Erhard M, Hervas J, Hiederer R, Jeffery S, Lukewille A, Marmo L, Montanarella L, Olazabal C, Petersen J, Penizek V, Strassburger T, Toth G, Van den Eeckhaut M, Van Liedekerke M, Verheijen F, Viestova E and Yigini Y, 2012. The state of soil in Europe. Joint Research Centre (European Commission) reference report. Available online at: http://ec.europa.eu/dgs/jrc//downloads/jrc_reference_report_2012_02_soil.pdf

Jordan M, 2001. Population dynamics of oribatid mites (Acari: Oribatida) on horse pastures of North Central Florida. M.Sc. Thesis, University of Florida, Unpublished 105 pp.

Joutey N, Wifak Bahafid W, Hanane Sayel H and N EG, 2013. Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms. In: *Biodegradation - Life of Science*. Eds Chamy R, Rosenkranz F, InTech, Rijeka, 289–320.

Kammenga JE, Dallinger R, Donker MH, Kohler HR, Simonsen V, Triebskorn R and Weeks JM, 2000. Biomarkers in terrestrial invertebrates for ecotoxicological soil risk assessment. In: *Reviews of Environmental Contamination and Toxicology, Vol 164: Continuation of Residue Reviews*. Ed Ware GW, 93–147.

Karpouzas DG, Kandeler E, Bru D, Friedel I, Auer Y, Kramer S, Vasileiadis S, Petric I, Udikovic-Kolic N, Djuric S and Martin-Laurent F, 2014. A tiered assessment approach based on standardized methods to estimate the impact of nicosulfuron on the abundance and function of the soil microbial community. Soil Biology and Biochemistry, 75, 282–291.

Kasiamdari R, Smith S, Smith F and Scott E, 2002. Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate *Rhizoctonia* and *Rhizoctonia solani* on mung bean (*Vigna radiata*). Plant and Soil, 238, 235–244.

Katagi T and Ose K, 2015. Toxicity, bioaccumulation and metabolism of pesticides in the earthworm. Journal of Pesticide Science, 40, 69–81.

Kattwinkel M, Römbke J and Liess M, 2012. Ecological recovery of populations of vulnerable species driving the risk assessment of pesticides. EFSA supporting publication 2012:EN-338, 98 pp.

Kattwinkel M, Liess M, Arena M, Bopp S, Streissl F and Römbke J, 2015. Recovery of aquatic and terrestrial populations in the context of European pesticide risk assessment. Environmental reviews, 23, 382–394.

Kauhala K and Ihalainen A, 2014. Impact of landscape and habitat diversity on the diversity of diets of two omnivorous carnivores. Acta Theriologica, 59, 1–12.

Kaya HK and Gaugler R, 1993. Entomopathogenic Nematodes. Annual Review of Entomology, 38, 181–206.

Kayang H, Sharma GD and Mishra RR, 1996. The influence of isopod grazing on microbial dynamics in decomposing leaf litter of *Alnus nepalensis* D. Don. European Journal of Soil Biology, 32, 35–39.

Ke X, Winter K and Filser J, 2005. Effects of soil mesofauna and farming management on decomposition of clover litter: a microcosm experiment. Soil Biology & Biochemistry, 37, 731–738.

Keith SA, Amrit FRG, Ratnappan R and Ghazi A, 2014. The *C. elegans* healthspan and stress-resistance assay toolkit. Methods, 68, 476–486.

Keller DA, Juberg DR, Catlin N, Farland WH, Hess FG, Wolf DC and Doerrer NG, 2012. Identification and Characterization of Adverse Effects in 21st Century Toxicology. Toxicological Sciences, 126, 291–297.

Kennelly M, O'Mara J, Rivard C, Miller G and Smith D, 2012. Introduction to abiotic disorders in plants. The Plant Health Instructor. doi: 10.1094/PHI-I-2012-10-29-01

Kerney M, Cameron R and Jungbluth J, 1983. *Die Landschnecken Nord-und Mitteleuropas*. Verlag Paul Parey, Hamburg, 384 pp.

Ketterings QM, Blair JM and Marinissen JCY, 1997. Effects of earthworms is on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. Soil Biology & Biochemistry, 29, 401–408.

King GKK, Larras F, Charles S and Delignette-Muller ML, 2015a. Hierarchical modelling of species sensitivity distribution: Development and application to the case of diatoms exposed to several herbicides. Ecotoxicology and Environmental Safety, 114, 212–221.

King GKK, Delignette-Muller ML, Kefford BJ, Piscart C and Charles S, 2015b. Constructing time-resolved Species Sensitivity Distributions using a hierarchical Toxico-Dynamic model. Environmental Science & Technology, 49, 12465–12473.

Knacker T, van Gestel CAM, Jones SE, Soares A, Schallnass HJ, Forster B and Edwards CA, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: conceptual approach and study design. Ecotoxicology, 13, 9–27.

Koch A, Kuhn G, Fontanillas P, Fumagalli L, Goudet J and Sanders I, 2004. High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. PNAS, 101, 2369–2374.

Koide R, Courty P and Garbaye J, 2007. Research perspectives on functional diversity in ectomycorrhizal fungi. New Phytologist, 174, 240–243.

Koide R, Fernandez C and Malcolm G, 2014. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. New Phytologist, 201, 433–439.

Köljalg U, Larsson K-H, Abarenkov K, Nilsson R, Alexander I, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor A, Tedersoo L, Vrålstad T and Ursing B, 2004. UNITE: a database providing web-based methods for thevmolecular identification of ectomycorrhizal fungi. New Phytologist, 166, 1063–1068.

Koppenhofer A, Cowles R, Cowles E, Fuzy E and Kaya H, 2003. Effect of neonicotinoid synergists on entomopathogenic nematode fitness. Entomologia Experimentalis Et Applicata, 106, 7–18.

Krab EJ, Oorsprong H, Berg MP and Cornelissen JHC, 2010. Turning northern peatlands upside down: disentangling microclimate and substrate quality effects on vertical distribution of Collembola. Functional Ecology, 24, 1362–1369.
Krab EJ, Cornelissen JHC and Berg MP, 2015. A simple experimental set-up to disentangle the effects of altered temperature and moisture regimes on soil organisms. Methods in Ecology and Evolution, 6, 1159–1168.
Kragten S, Tamis WLM, Gertenaar E, Ramiro SMM, Van der Poll RJ, Wang J and De Snoo GR, 2011. Abundance of invertebrate prey for birds on organic and conventional arable farms in the Netherlands. Bird Conservation International, 21, 1–11.
Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, Grossart H-P, Philippot L and Bodelier PLE, 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology, 5, 251.
Kreutzweiser D, Good K, Chartrand D, Scarr T, Holmes S and Thompson D, 2008. Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. Pest Management Science, 64, 112–118.
Krogh PH, 1995. Does a heterogeneous distribution of food or pesticide affect the outcome of toxicity tests with collembolan? Ecotoxicology and Environmental Safety, 30, 158–163.
Krogh P, 2008. Toxicity testing with the collembolans *Folsomia fimetaria* and *Folsomia candida* and the results of a ringtest. Danish Environmental Protection Agency, Environmental Project No. 1256.
Kruuk H and Parish T, 1985. Food, food availability and weight of badgers (*Meles meles*) in relation to agricultural changes. Journal of Applied Ecology, 22, 705–715.
Kuchenbuch R and Ingram K, 2004. Effects of soil bulk density on seminal and lateral roots of young maize plants (*Zea mays* L.). Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, 167, 229–235.
Kula C, Heimbach F, Riepert F and Römbke J, 2006. Technical recommendations for the update of the ISO earthworm field test guideline (ISO 11268-3). Journal of Soils and Sediments, 6, 182–186.
Kull CA, de Sartre XA and Castro-Larranaga M, 2015. The political ecology of ecosystem services. Geoforum, 61, 122–134.
Kuusk A-K and Ekbom B, 2010. Lycosid spiders and alternative food: feeding behavior and implications for biological control. Biological Control, 55, 20–26.
Lagerlof J, Goffre B and Vincent C, 2002. The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agriculture Ecosystems & Environment, 89, 91–103.
Lagerlof J, Insunza V, Lundegardh B and Ramert B, 2011. Interaction between a fungal plant disease, fungivorous nematodes and compost suppressiveness. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 61, 372–377.
Laine-Kaulio H, Backnäs S, Koivusalo H and Laurén A, 2015. Dye tracer visualization of flow patterns and pathways in glacial sandy till at a boreal forest hillslope. Geoderma, 259–260, 23–34.
Landa ER and Feller C, 2009. *Soil and Culture*. Springer, The Netherlands 525 pp.
Landi S, Manachini B, Valentini M, Jucker C and Colombo M, 2009. *Pristionchus* sp. (Rhabditida: diplogastridae) from Italian populations of Anoplophora chinensis Forster (Coleoptera: cerambycidae). IOBC/WPRS Bulletin, 45, 413–416.
Lange OL, Nobel PS, Osmond BC and Ziegler H, 2012. *Physiological Plant Ecology IV: ecosystem Processes: mineral Cycling, Productivity and Man's Influence*. Spriger-Verlag, Berlin, Germany. 646 pp.
Langmaack M, Schrader S, Rapp-Bernhardt U and Kotzke K, 1999. Quantitative analysis of earthworm burrow systems with respect to biological soil-structure regeneration after soil compaction. Biology and Fertility of Soils, 28, 219–229.
Larink O and Schrader S, 1999. How to investigate the mutual influence of soil animals and soil compaction. In: van den Akker J, Arvidson J and Horn R (eds). *Experiences with the Impact and Prevention of Subsoil Compaction in the European Community*. Wageningen, DLO Winand Staring Centre. pp. 171–179.
Larink O and Schrader S, 2003. Hoher Druck und die Luft ist raus - Bodenverdichtung, Bodentiere und nachhaltige Landwirtschaft. 1/2003.
Larink O, Werner D, Langmaack M and Schrader S, 2001. Regeneration of compacted soil aggregates by earthworm activity. Biology and Fertility of Soils, 33, 395–401.
Larochelle A, 1972. Notes on the food of *Cychrini* (Coleoptera: carabidae). The Great Lakes Entomologist, 5, 81–83.
Larsen T, Schjonning P and Axelsen J, 2004. The impact of soil compaction on euedaphic Collembola. Applied Soil Ecology, 26, 273–281.
Lartey RT, Curl EA and Peterson CM, 1994. Interactions of mycophagous collembola and biological control fungi in the suppression of *Rhizoctonia solani*. Soil Biology and Biochemistry, 26, 81–88.
Lavelle P, 2002. Functional domains in soils. Ecological Research, 17, 441–450.
Lavelle P and Spain AV (eds), 2005. *Soil Ecology*. Springer, Dordrecht, The Netherlands. 654 pp.
Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P and Rossi JP, 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology, 42, S3–S15.
Lee K and Foster R, 1991. Soil fauna and soil structure. Australian Journal of Soil Research, 29, 745–775.

Lehmann A and Rillig MC, 2015. Understanding mechanisms of soil biota involvement in soil aggregation: a way forward with saprobic fungi. Soil Biology & Biochemistry, 88, 298–302.
Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC and Schleper C, 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature, 442, 806–809.
Lever J, van Nes E, Scheffer M and Bascompte J, 2014. The sudden collapse of pollinator communities. Ecology Letters, 17, 350–359.
Liess M and Von der Ohe PC, 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry, 24, 954–965.
Liess M, Foit K, Becker A, Hassold E, Dolciotti I, Kattwinkel M and Duquesne S, 2013. Culmination of low-dose pesticide effects. Environmental Science and Technology, 47, 8862–8868.
Lilleskov E and Bruns T, 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia, 97, 762–769.
Litterick A, Harrier L, Wallace P, Watson C and Wood M, 2004. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production - A review. Critical Reviews in Plant Sciences, 23, 453–479.
Liu Y, Zaprasis A, Liu S, Drake H and Horn M, 2011. The earthworm *Aporrectodea caliginosa* stimulates abundance and activity of phenoxyalkanoic acid herbicide degraders. International Society for Microbial Ecology Journal, 5, 473–485.
Lokke H and Van Gestel CAM (eds), 1998. *Handbook of Soil Invertebrate Toxicity Tests*. John Wiley & Sons, Chichester. 251 pp.
Loureiro S, Soares A and Nogueira AJA, 2005. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution, 138, 121–131.
Loureiro S, Amorim MJB, Campos B, Rodrigues SMG and Soares AMVM, 2009. Assessing joint toxicity of chemicals in *Enchytraeus albidus* (Enchytraeidae) and *Porcellionides pruinosus* (Isopoda) using avoidance behaviour as an endpoint. Environmental Pollution, 157, 625–636.
Luck GW, Daily GC and Ehrlich PR, 2003. Population diversity and ecosystem services. Trends in Ecology and Evolution, 18, 331–336.
Lummer D, Scheu S and Butenschoen O, 2012. Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. Oikos, 121, 1649–1655.
Ma W and Bodt J, 1993. Differences in Toxicity of the Insecticide Chlorpyriphos to 6 Species of Earthworms (Oligochaeta, Lumbricidae) in Standardized soil tests. Bulletin of Environmental Contamination and Toxicology, 50, 864–870.
Ma W and Imerzeel B, 1995. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenathrene and fluoranthene. Ecotoxicology and Environmental Safety, 32, 226–232.
Ma Q, Gan J, Becker JO, Papiernik SK and Yates SR, 2001. Evaluation of propargyl bromide for control of barnyardgrass and Fusarium oxysporum in three soils. Pest Management Science, 57, 781–786.
Maaß S, Caruso T and Rillig MC, 2015. Functional role of microarthropods in soil aggregation. Pedobiologia, 58, 59–63.
Mack K, Vollmer T, Illig J, Appeltauer A and Knaebe S, 2016. MDDs in micro-arthropod field testing. Poster. SETAC Conference, Nantes, France.
Mackay D, 1988. On low, very low, and negligible concentrations. Environmental. Toxicology and. Chemistry, 7, 1–3.
Malherbe S and Marais D, 2015. Nematode community profiling as a soil biology monitoring tool in support of sustainable tomato production: a case study from South Africa. Applied Soil Ecology, 93, 19–27.
Mallawatantri A, McConkey B and Mulla D, 1996. Characterization of pesticide sorption and degradation in macropore linings and soil horizons of Thatuna silt loam. Journal of Environmental Quality, 25, 227–235.
Mallmann GC, 2016. Pollutant effect on arbuscular mycorrhizal fungi: proposal for the adaptation and expansion of use of the protocol ISO/TS 10832:2009.2016. MSc Dissertation in Soil Science, University of the State of Santa Catarina, 112 pp.
Manachini B, 2013. Pesticides: chemical and biological. In: *Encyclopedia of Environmental Management*. Jorgenson SE (ed.). Taylor and Francis, New York, 1986–1996.
Maraun M and Scheu S, 1996. Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by millipedes (*Glomeris marginata*). Oecologia, 107, 131–140.
Mariani L, Jimenez J, Asakawa N, Thomas R and Decaens T, 2007. What happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in Neotropical savannahs. Soil Biology & Biochemistry, 39, 757–767.
Marschner H and Dell B, 1994. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil, 159, 89–102.
Martin E, Reineking B, Seo B and Steffan-Dewenter I, 2013. Natural enemy interactions constrain pest control in complex agricultural landscapes. Proceedings of the National Academy of Sciences of the United States of America, 110, 5534–5539.
Martins da Silva P, Berg M, Serrano ARM, Dubs F and Sousa JP, 2012. Environmental factors at different spatial scales governing soil fauna community patterns in fragmented forests. Landscape Ecology, 27, 1337–1349.
Mason C, 1970. Food, feeding rates and assimilation in woodland snails. Oecologia, 5, 215–239.

Mathieu J, Barot S, Blouin M, Caro G, Decaens T, Dubs F, Dupont L, Jouquet P and Nai P, 2010. Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, *Aporrectodea icterica* and *Dendrobaena veneta*, in a mesocosm experiment. Soil Biology & Biochemistry, 42, 203–209.
McBrayer JF and Reichle DE, 1971. Trophic structure and feeding rates of forest soil invertebrate populations. Oikos, 22, 381–388.
MEA (Millennium Ecosystem Assessment), 2005. Ecosystems and human well-being: Biodiversity synthesis 2005. World Resources Institute, Washington. DC. Ecological Management & Restoration, 6(1442–7001), 226–227.
Meharg A, 1996. Bioavaibility of Atrazine to soil microbes in the presence of the earthworm *Lumbricus terrestrius* (L.). Soil Biology & Biochemistry, 28, 555–559.
Meli M, Auclerc A, Palmqvist A, Forbes VE and Grimm V, 2013. Population-level consequences of spatially heterogeneous exposure to heavy metals in soil: an individual-based model of springtails. Ecological Modelling, 50, 338–351.
Meli M, Palmqvist A, Forbes VE, Groeneveld J and Grimm V, 2014. Two pairs of eyes are better than one: combining individual-based and matrix models for ecological risk assessment of chemicals. Ecological Modelling, 280, 40–52.
Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider J, Piceno Y, DeSantis T, Andersen G, Bakker P and Raaijmakers J, 2011. Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. Science, 332, 1097–1100.
Metz LJ, 1971. Vertical movement of Acarina under moisture gradients. Pedobiologia, 11, 262–268.
Millard SP and Bross ID, 1987. Proof of safety vs. proof of hazard. Biometrics, 43, 719–725.
Mitchell MJ, 1978. Vertical and horizontal distributions of Oribatid mites (Acari: Cryptostigmata) in an aspen woodland soil. Ecology, 59, 516–525.
Mod HK, le Roux PC, Guisan A and Luoto M, 2015. Biotic interactions boost spatial models of species richness. Ecography, 38, 913–921.
Monroy F, Aira M, Gago J and Domínguez J, 2007. Life cycle of the earthworm Octodrilus complanatus (Oligochaeta, Lumbricidae). Comptes Rendus Biologies, 330, 389–391.
Moreby S, Aebischer N, Southway S and Sotherton N, 1994. A comparison of the flora and arthropod fauna of organically grown winter wheat in Southern England. Annals of Applied Biology, 125, 13–27.
Morgado RG, Gomes PAD, Ferreira NGC, Cardoso DN, Santos MJG, Soares AMVM and Loureiro S, 2016. Toxicity interaction between chlorpyrifos, mancozeb and soil moisture to the terrestrial isopod *Porcellionides pruinosus*. Chemosphere, 144, 1845–1853.
Moser T, Schallnaß HJ, Jones SE, Van Gestel CAM, Koolhaas JE, Rodrigues JML and Römbke J, 2004a. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on nematodes. Ecotoxicology, 13, 61–74.
Moser T, Van Gestel CAM, Jones SE, Koolhaas JE, Rodrigues JML and Römbke J, 2004b. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – An instrument for testing potentially harmful substances: effects of carbendazim on enchytraeids. Ecotoxicology, 13, 89–103.
Mouillot D, Bellwood DR, Baraloto C, Chave J, Galzin R, Harmelin-Vivien M, Kulbicki M, Lavergne S, Lavorel S, Mouquet N, Paine CET, Renaud J and Thuiller W, 2013. Rare Species Support Vulnerable Functions in High-Diversity Ecosystems. PLoS Biology, 11, 1–11.
Mueller K and Deurer M, 2011. Review of the remediation strategies for soil water repellency. Agriculture Ecosystems & Environment, 144, 208–221.
Mukerji K and Ciancio A, 2007. *General Concepts in Integrated Pest and Disease Management*. Ciancio A, Mukerji KG (eds). Springer, Amsterdam, 359 pp.
Mulder C, Cohen JE, Setala H, Bloem J and Breure AM, 2005. Bacterial traits, organism mass, and numerical abundance in the detrital soil food web of Dutch agricultural grasslands. Ecology Letters, 8, 80–90.
Munkvold L, Kjøller R, Vestberg M, Rosendahl S and Jakobsen I, 2004. High functional diversity within species of arbuscular mycorrhizal fungi. New Phytologist, 164, 357–364.
Munns WR Jr, Gervais J, Hoffman AA, Hommen U, Nacci DR, Nakamaru M, Sibly R and Topping CJ. 2008. Modeling approaches to population-level risk assessment. In: *Population-Level Ecological Risk Assessment*. Eds Barnthouse LW, Munns WR Jr, Sorensen MT. CRC Press, Boca Raton, FL, 179–210.
Munns WR, Rea AW, Suter GW, Martin L, Blake-Hedges L, Crk T, Davis C, Ferreira G, Jordan S, Mahoney M and Barron MG, 2016. Ecosystem services as assessment endpoints for ecological risk assessment. Integrated Environmental Assessment and Management, 12, 522–528.
Naeem S, 1998. Species redundancy and ecosystem reliability. Conservation Biology, 12, 39–45.
Naeem S and Li SB, 1997. Biodiversity enhances ecosystem reliability. Nature, 390, 507–509.
Natal-da-Luz T, Lee I, Verweij RA, Morais PV, Van Velzen MJ, Sousa JP and Van Gestel CA, 2012. Influence of earthworm activity on microbial communities related with the degradation of persistent pollutants. Environmental Toxicology and Chemistry, 31, 794–803.
National Research Council, 2000. Natural Attenuation for Groundwater Remediation.
Natori Y and Chenoweth R, 2008. Differences in rural landscape perceptions and preferences between farmers and naturalists. Journal of Environmental Psychology, 28, 250–267.
Neher DA, 2001. Role of Nematodes in Soil Health and Their Use as Indicators. Journal of Nematology, 33, 161–168.

Neher D, Weicht T and Barbercheck M, 2012. Linking invertebrate communities to decomposition rate and nitrogen availability in pine forest soils. Applied Soil Ecology, 54, 14–23.
Newell P, 1967. Molluscs. In: Burgers A and Raw F (ed.). *Soil Biology*. Academic Press, London. p. 20.
Ninh HT, Grandy AS, Wickings K, Snapp SS, Kirk W and Hao J, 2015. Organic amendment effects on potato productivity and quality are related to soil microbial activity. Plant and Soil, 386, 223–236.
Nishimatsu T and Jackson JJ, 1998. Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology, 91, 410–418.
Noble R and Coventry E, 2005. Suppression of soil-borne plant diseases with composts: A review. Biocontrol Science and Technology, 15, 3–20.
Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R and Levin SA, 2001. Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. Proceedings of the National Academy of Sciences of the United States of America, 98, 11376–11381.
Novais SC, Soares AMVM and Amorim MJB, 2010. Can avoidance in *Enchytraeus albidus* be used as a screening parameter for pesticides testing? Chemosphere, 79, 233–237.
Nuutinen V, Butt KR and Jauhiainen L, 2011. Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population. Pedobiologia, 54, S167–S172.
Nyffeler M and Symondson W, 2001. Spiders and harvestmen as gastropod predators. Ecological Entomology, 26, 617–628.
Odling-Smee F, Laland K and Feldman M, 2003. Niche construction: the neglected process in evolution.
OECD (Organization for Economic Co-operation and Development), 2000. Soil Microorganisms: Nitrogen Transformation Test (No. 216). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2002. Aerobic and anaerobic transformation in soil (No 307). OECD guideline for the testing of chemicals. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2004. Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (No. 222). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2006. Guidance document on the breakdown of organic matter in litter bags. Oecd series on testing and assessment, No 56. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2008. Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer*) reproduction test in soil (No. 226). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2009. Collembolan Reproduction Test in Soil (No. 232). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
OECD (Organization for Economic Co-operation and Development), 2010. Bioaccumulation in Terrestrial Oligochaetes (No. 317). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
Oldenburg E, Kramer S, Schrader S and Weinert J, 2008. Impact of the earthworm *Lumbricus terrestris* on the degradation of Fusarium-infected and deoxynivalenol-contaminated wheat straw. Soil Biology and Biochemistry, 40, 3049–3053.
Olsson PA, Jakobsen I and Wallander H, 2002. Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environ- ment. In: Van der Heijden MGA and Sanders I (eds.). *Mycorrhizal Ecology*. Springer-Verlag, Berlin. pp. 93–115.
Ortega-Calvo J, Harmsen J, Parsons J, Semple K, Versonnen B, Aitken M, Ajao C, Eadsforth C, Galay-Burgos M, Naidu R, Oliver R, Peijnenburg W, Römbke J and Streck G, 2015. From bioavailability science to regulation of organicbchemicals. Environmental Science and Technology, 49, 10255–10264.
Osler G and Sommerkorn M, 2007. Toward a complete soil C and N cycle: incorporating the soil fauna. Ecology, 88, 1611–1621.
Ouvrard S, Chenot E, Masfaraud J and Schwartz C, 2013. Long-term assessment of natural attenuation: statistical approach on soils with aged PAH contamination. Biodegradation, 24, 539–548.
Owojori OJ, Healey J, Princz J and Siciliano SD, 2011. Can Avoidance Behavior of the mite Oppia Nitens be used as a rapid toxicity test for soils contaminated with metals or organic chemicals? Environmental Toxicology and Chemistry, 30, 2594–2601.
Owojori O, Waszak K and Römbke J, 2014. Avoidance and reproduction tests with the predatory mite Hyapoaspis Aculeifer:effects of different chemical substances. Environmental Toxicology and Chemistry, 33, 230–237.
Pace NR, 2009. Mapping the Tree of Life: progress and Prospects. Microbiology and Molecular Biology Reviews, 73, 565–576.
Palmer MA, Ambrose RF and Poff NL, 1997. Ecological theory and community restoration ecology. Restoration Ecology, 5, 291–300.
Parkhurst DF, 2001. Statistical significance tests: equivalence and reverse tests should reduce misinterpretation. BioScience, 5, 1051–1057.
Peijnenburg W, Capri E, Kula C, Liess M, Luttik R, Montforts M, Nienstedt K, Römbke J, Sousa JP and Jensen J, 2012. Evaluation of exposure metrics for effect assessment of soil invertebrates. Critical Reviews in Environmental Science and Technology, 42, 1862–1893.
Pell M, Stenberg B and Torstensson L, 1998. Potential denitrification and nitrification tests for evaluation of pesticide effects in soil. Ambio, 27, 24–28.

Pelosi C, Joimel S and Makowski D, 2013. Searching for a more sensitive earthworm species to be used in pesticide homologation tests - A meta-analysis. Chemosphere, 90, 895–900.
Pelosi C, Barot S, Capowiez Y, Hedde M and Vandenbucke F, 2014. Pesticides and earthworms. A review. Agronomy for Sustainable Development, 34, 199–228.
Pelosi C, Bertrand M, Thénard J and Mougin C, 2015. Earthworms in a 15 years agricultural trial. Applied Soil Ecology, 88, 1–8.
Peng S, Guo T and Liu G, 2013. The effects of arbuscular mycorrhizal hyphal networks on soil aggregations of purple soil in southwest China. Soil Biology & Biochemistry, 57, 411–417.
Pereg L and McMillan M, 2015. Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biology & Biochemistry, 80, 349–358.
Persmark L, MarbanMendoza N and Jansson HB, 1995. Nematophagous fungi from agricultural soils of Central America. Nematropica, 25, 117–124.
Petersen H and Luxton M, 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos, 39, 288–388.
Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones C, Sarr A and Maron P, 2013. Loss in microbial diversity affects nitrogen cycling in soil. Isme Journal, 7, 1609–1619.
Pieper S and Weigmann G, 2008. Interactions between isopods and collembolans modulate the mobilization and transport of nutrients from urban soils. Applied Soil Ecology, 39, 109–126.
Pieper S, Mohnke O, Prokoph K, Wessolek K and Weigmann G, 2008. Characterization of earthworm impact on water distribution and nutrient dynamics in urban soils. Bodenbiologische Indikatoren für eine nachhaltige Bodennutzung. Available online: http://eprints.dbges.de/id/eprint/34
Pieper S, Egerer S, Kotschik P, Sole M and Scholz-Starke B, 2016. Multispecies tests with soil organisms. Proceedings of the Workshop 2014 Dessau, 31 pp.
Porto ALM, Melgar G, Kasemodel C and Nitschke M, 2011. Biodegradation of pesticides. In: *Pesticides in the Modern World - Pesticides Use and Management*. Ed Stoycheva M, InTech, 407–438.
Poßberg C, Hammers-Wirts M, Toschki A and Schäffer A, 2014. Fate of lindane and imidacloprid in terrestrial model ecosystems (TMEs) during a sampling period of one year. Proceedings of the 24th SETAC Europe Annual Meeting, Basel, Switzerland.
Postma-Blaauw M, Bloem J, Faber JH, van Groenigen J, de Goede R and Brussaard L, 2006. Earthworm species composition affects the soil bacterial community and net nitrogen mineralization. Pedobiologia, 50, 243–256.
Princz JI, Behan-Pelletier VM, Scroggins RP and Siciliano SD, 2010. Oribatid mites in soil toxicity testing-The use of Oppia Nitens (CL KOCK) as a new test species. Environmental Toxicology and Chemistry, 29, 971–979.
Prinzing A, Kretzler S, Badejo A and Beck L, 2002. Traits of oribatid mite species that tolerate habitat disturbance due to pesticide application. Soil Biology and Biochemistry, 34, 1655–1661.
Prosser J, 2007. Microorganisms cycling soil nutrients and their diversity. In: Van Elsas JD, Jansson JK and Trevors JT (eds). *Modern soil microbiology*, 2nd edition. CRC Press, Boca Raton, FL. pp. 237–261.
Puglisi E, 2012. Response of microbial organisms (aquatic and terrestrial) to pesticides. EFSA Supporting Publications 2012:EN-359, 175 pp.
Rantalainen M, Fritze H, Haimi J, Pennanen T and Setala H, 2005. Colonisation of newly established habitats by soil decomposer organisms: the effect of habitat corridors in relation to colonisation distance and habitat size. Applied Soil Ecology, 28, 67–77.
Rautmann D, Streloke M and Winkler R, 2001. New basic drift values in the authorisation procedure for plant protection products. Proceedings of the Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorisation of Plant Protection Products (WORMM), 133-141.
Rebel K, Phillips RP, Fransson P and Brzostek E, 2013. The role of mycorrhizal fungi in integrated carbon and nitrogen cycles. Geophysical Research Abstracts, 15, EGU2013-10746.
Reed M, Alvarez T, Chelinho S, Forbes V, Johnston A, Meli M, Voss F and Pastorok R, 2016. A Risk Assessment Example for Soil Invertebrates Using Spatially Explicit Agent-Based Models. Integrated Environmental Assessment and Management, 12, 58–66.
Reichenberger S, Bach M and Frede H, 2007. Auswertung der prädiktiven PRZM-Simulationen aus Reichenberger (2005) zum Austrag von PSM-Wirkstoffen über Runoff und Erosion in Deutschland. Dokumentation für den Industrieverband Agrar (IVA). Finale Version vom 10.08.2007.
Renaud JM, 2012. What more can avoidance data tell us? Using avoidance assays as a tool to predict the recolonization potential of contaminated sites. M.Sc. thesis. University of Coimbra. 53 pp.
Reuter HI and Eden M, 2008. Agriculture Field Shape Parameters on NUTS-3 level for Wind erosion research estimated based on the Image2000 archive. Available online: http://esdac.jrc.ec.europa.eu/themes/agriculture-field-parameters
Ribeiro S, Sousa JP, Nogueira AJA and Soares AMVM, 2001. Effect of Endosulfan and Parathion on Energy Reserves and Physiological Parameters of the Terrestrial Isopod *Porcellio dilatatus*. Ecotoxicology and Environmental Safety, 49, 131–138.
Ricci PF, Cox LA and Baram M, 1987. De minimis considerations in health risk assessment. Journal of Hazardous Materials, 15, 77–95.

Rillig MC, Wright SF, Kimball BA, Pinter PJ, Wall GW, Ottman MJ and Leavitt SW, 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a Sorghum field: a possible role for arbuscular mycorrhizal fungi. Global Change Biology, 7, 333–337.
Rillig MC, Wright SF and Eviner VT, 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil, 238, 325–333.
Rillig MC, Aguilar-Trigueros CA, Bergmann J, Verbruggen E, Veresoglou SD and Lehmann A, 2015. Plant root and mycorrhizal fungal traits for understanding soil aggregation. New Phytologist, 205, 1385–1388.
Ritsema C and Dekker L, 2000. Preferential flow in water repellent sandy soils: principles and modeling implications. Journal of Hydrology, 231, 308–319.
Rittmann B, 2004. Definition, objectives, and evaluation of natural attenuation. Biodegradation, 15, 349–357.
Rodríguez H and Fraga R, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances, 17, 319–339.
Rogge E, Nevens F and Gulinck H, 2007. Perception of rural landscapes in Flanders: looking beyond aesthetics. Landscape and Urban Planning, 82, 159–174.
Roithmeier O and Pieper S, 2009. Influence of Enchytraeidae (*Enchytraeus albidus*) and compaction on nutrient mobilization in an urban soil. Pedobiologia, 53, 29–40.
Römbke J, 2008. Bioavailability in soil: the role of invertebrate behaviour. In: *Chemical Bioavailability in Terrestrial Environments. Developments in Soil Science*. Ed Naidu R, Bolan NS, Megharaj M, Juhasz AI, Gupta SK, Clothier BE, Schulin R. Elsevier, Amsterdam, The Netherlands, 245–260.
Römbke J, Van Gestel CAM, Jones SE, Koolhaas JE, Rodrigues JML and Moser T, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on earthworms. Ecotoxicology, 13, 105–118.
Römbke J, Hanagarth W, Höfer H, Garcia M and Martius C, 2006. Feeding rates of soil organisms at four different forest sites in Amazonia. Journal of Tropical Ecology, 22, 313–320.
Römbke J, Schmelz R.M., Knäbe S., 2009. Field studies for the assessment of pesticides with soil mesofauna, in particular enchytraeids, mites, and nematodes: Design and first results Soil Organisms, 81, 237–264.
Römbke J, Schmelz R.M., Knöbefer S., 2009. Field studies for the assessment of pesticides with soil mesofauna, in particular enchytraeids, mites, and nematodes: Design and first results. Soil Organisms, 81, 237–264.
Römbke J, Jänsch S, Murillo L and Sousa JP, 2010. Further development of a strategy for the assessment of the risk of plant protection products to soil organisms – considering different life form types and exposure scenarios. Report FKZ 360 03 047 for the German Federal Environmental Agency (UBA).
Römbke J, Jänsch S, Roß-Nickoll M, Toschki A, Höfer H, Horak F, Russell D, Burkhardt U and Schmitt H, 2012. Erfassung und Analyse des Bodenzustands im Hinblick auf die Umsetzung und Weiterentwicklung der Nationalen Biodiversitätsstrategie. UBA Texte. 33/2012, 386 pp.
Romero SA and Harwood JD, 2010. Diel and seasonal patterns of prey available to epigeal predators: evidence for food limitation in a linyphiid spider community. Biological Control, 52, 84–90.
Rorke MA, Gardner DR and Greenhalgh R, 1974. Lethality and behavioural symptoms produced by some organophosphorous compounds in the snail (Helix aspersa). Bulletin of environmental contamination and toxicology, 11, 417–424.
Rubach MN, Ashauer R, Buchwalter DB, De Lange HJ, Hamer M, Preuss TG, Töpke K and Maund SJ, 2011. Framework for traits-based assessment in ecotoxicology. Integrated Environmental Assessment and Management, 7, 172–186.
Rutgers M, Mulder C, Schouten AJ, Bloem J, Bogte JJ, Breure AM, Brussaard L, de Goede RGM, Faber JH, Jagers op Akkerhuis GAJM, Keidel H, Korthals GW, Smeding FW, ten Berg C and van Eekeren N, 2008. Soil ecosystem profiling in the Netherlands with ten references for biological soil quality. RIVM-Report 607604009, 85 pp.
Sabatini M and Innocenti G, 2001. Effects of Collembola on plant-pathogenic fungus interactions in simple experimental systems. Biology and Fertility of Soils, 33, 62–66.
Santos S, Sousa JP, Frost M and Soares AMVM, 2003. Time-dependent toxicokinetics of (14)C lindane in the terrestrial isopod *Porcellionides pruinosus*. Environmental Toxicology and Chemistry, 22, 2221–2227.
Santos MJG, Soares AMVM and Loureiro S, 2010. Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida*. Chemosphere, 80, 1021–1030.
Schäfer RB, Caquet T, Siimes K, Mueller R, Lagadic L and Liess M, 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment, 382, 272–285.
Schäffer A, Van den Brink P, Heimbach F, Hoy SP, de Jong F, Römbke J, Sousa JP and Ross-Nickoll M, 2008. Semi-field methods are a useful tool for the environmental risk assessment of pesticides in soil – Developed on the discussions at the SETAC workshop PERAS, Coimbra, Portugal, October 2007. Environmental Science and Pollution Research, 15, 176–177.
Schäffer A, van den Brink P, Heimbach F, Hoy SP, de Jong F, Römbke J, Ross-Nickoll M and Sousa JP, 2010. Semi-field methods for the environmental risk assessment of pesticides in soil. Eds Schäffer A, Brink PJvd, Heimbach F, Hoy SP, Jong FMWd, Römbke J, Ross-Nickoll M, Sousa JP. CRC Press, Boca Raton, FL, 105 pp.
Schjønning P, Thomsen IK and Christensen BT, 1998. Soil porosity as a habitat for microorganisms. In: *NJF-UTREDNING/rapport*. Ed Børresen T, 49–56.

Schmitt T, Louy D, Zimmermann E and Habel JC, 2016. Species radiation in the Alps: multiple range shifts caused diversification in Ringlet butterflies in the European high mountains. Organisms, Diversity and Evolution, 16, 791–808.
Schneider K, Renker C and Maraun M, 2005. Oribatid mite (Acari, Oribatida) feeding on ectomycorrhizal fungi. Mycorrhiza, 16, 67–72.
Schneider S, Widmer F, Jacot K, Kölliker R and Enkerli J, 2012. Spatial distribution of Metarhizium clade 1 in agricultural landscapes with arable land and different semi-natural habitats. Applied Soil Ecology, 52, 20–28.
Schnug L, Jensen J, Scott-Fordsmand JJ and Leinaas HP, 2014. Toxicity of three biocides to springtails and earthworms in a soil multi-species (SMS) test system. Soil Biology & Biochemistry, 74, 115–126.
Scholz-Starke B, 2013. Assessing the risks of pesticides to soil communities using terrestrial model ecosystems. University of Aachen, Thesis 291 pp.
Scholz-Starke B, 2016. Analysis of existing laboratory studies with earthworms: Influence of study design variants on ecotoxicological test results of plant protection and veterinary pharmaceuticals. Federal Environment Agency Umweltbundesamt. FKZ 93 104 417 final report.
Scholz-Starke B, Beylich A, Moser T, Nikolakis A, Rumpler N, Schäffer A, Theißen B, Toschki A and Roß-Nickoll M, 2013. The response of soil organism communities to the application of the insecticide lindane in terrestrial model ecosystems. Ecotoxicology, 22, 339–362.
Schrader S and Zhang H, 1997. Earthworm casting: stabilization or destabilization of soil structure? Soil Biology & Biochemistry, 29, 469–475.
Schrader S, Joschko M, Kula H and Larink O, 1995. Earthworm effects on soil structure with emphasis on soil stability and soil water movement. In: *oil Structure—Its Development and Function, Advances in Soil Science*. Eds Hartge K, BA S, CRC Press, Boca Raton.
Schrader S, Langmaack M and Helming K, 2001. Effect of mesofaunal activity on the rehabilitation of sealed soil surfaces. Applied Soil Ecology, 16, 121–130.
Schrader S, Wolfarth F and Oldenburg E, 2013. Biological control of soil-borne phytopathogenic fungi and their mycotoxins by soil fauna – a review. Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 70, 291–298.
Schroter M, van der Zanden EH, van Oudenhoven APE, Remme RP, Serna-Chavez HM, de Groot RS and Opdam P, 2014. Ecosystem services as a contested concept: a synthesis of critique and counter-arguments. Conservation Letters, 7, 514–523.
Schuytema GS, Nebeker AV and Griffis WL, 1994. Effects of dietary exposure to forest pesticides on the brown garden snail Helix Aspersa Muller. Archives of Environmental Contamination and Toxicology, 26, 23–28.
Setala H, 2002. Sensitivity of ecosystem functioning to changes in trophic structure, functional group composition and species diversity in belowground food webs. Ecological Research, 17, 207–215.
Sheehan C, Kirwan L, Connolly J and Bolger T, 2008. The effects of earthworm functional diversity on microbial biomass and the microbial community level physiological profile of soils. European Journal of Soil Biology, 44, 65–70.
Sheenan C, Kriwan L, Connolly J and Bolger T, 2007. The effects of earthworm functional group diversity on earthworm community structure. Pedobiologia, 50, 479–487.
Sheppard SC, Bembridge JD, Holmstrup M and Posthuma L, 1998. Advances in earthworm ecotoxicology. Eds Sheppard SC, Bembridge JD, Holmstrup M, Posthuma L. SETAC, Amsterdam, 472 pp.
Shipitalo M, Edwards W and Redmond C, 1994. Comparison of water movement and quality in earthworm burrows and pan lysimeters. Environmental Quality, 23, 1345–1351.
Shukla M, 2014. *Soil Physics: an introduction*. CRC Press, Taylor & Francis Group 478 pp.
Shuttleworth K and Cerniglia C, 1995. Environmental aspects of PAH biodegradation. Applied Biochemistry and Biotechnology, 54, 291–302.
Siddiky MRK, Schaller J, Caruso T and Rillig MC, 2012. Arbuscular mycorrhizal fungi and collembola non-additively increase soil aggregation. Soil Biology & Biochemistry, 47, 93–99.
Simon M, Schlechtriem C, Römbke J, Egeler P and Weil M, 2016. Draft Report, Accumulation of contaminants in soil organisms – deduction and verification of suitable criteria for assessment of accumulation of contaminants in terrestrial organisms (terrestrial bioaccumulation), research project of the German Environment Agency (UBA), FKZ 371364419, Unpublished data.
Singh BK, Quince C, Macdonald CA, Khachane A, Thomas N, Abu Al-Soud W, Sorensen SJ, He Z, White D, Sinclair A, Crooks B, Zhou J and Campbell CD, 2014. Loss of microbial diversity in soils is coincident with reductions in some specialized functions. Environmental Microbiology, 16, 2408–2420.
Šmídová K and Hofman J, 2014. Uptake kinetics of five hydrophobic organic pollutants in the earthworm *Eisenia fetida* in six different soils. Journal of Hazardous Materials, 267, 175–182.
Smith V and Bradford M, 2003. Do non-additive effects on decomposition in litter-mix experiments result from differences in resource quality between litters? Oikos, 102, 235–242.
Smith SE and Read DJ. 2008. *Mycorrhizal symbiosis*. Amsterdam, the Netherlands, Academic Press.
Smith S, Jakobsen I, Grønlund M and Smith F, 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiologist, 156, 1050–1057.

Sochova I, Hofman J and Holoubek I, 2007. Effects of seven organic pollutants on soil nematode *Caenorhabditis elegans*. Environment International, 33, 798–804.
Soil Science Society of America, 2008. *Glossary of Soil Science Terms*. SSA, Madison, WI, USA.
Soka G and Ritchie M, 2014. Arbuscular mycorrhizal symbiosis and ecosystem processes: prospects for future research in tropical soils. Open Journal of Ecology, 4, 11–22.
Solomon K, Brock T, DeZwart D, Dyer SD, Postthuma L, Richards S, Sanderson H, Sibley P and VanDenBrink P, 2008. Extrapolation Practice for Ecotoxicological effect Characterization of Chemicals. Eds Solomon K, Brock T, DeZwart D, Dyer SD, Postthuma L, Richards S, Sanderson H, Sibley P, VanDenBrink P. CRC Press, Boca Raton, FL, 408 pp.
Sousa JP, Loureiro S, Pieper S, Frost M, Kratz W, Nogueira A and Soares A, 2000. Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod. Environmental Toxicology and Chemistry, 19, 2557–2563.
Sousa JP, Rodrigues JML, Loureiro S, Soares A, Jones SE, Forster B and Van Gestel CAM, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on soil microbial parameters. Ecotoxicology, 13, 43–60.
Southcombe E, Miller P, Ganzelmeier H, van de Zande J, Miralles A and Hewitt A, 1997. The international (BCPC) spray classification system including drift potential factor. Proceedings of 371–380.
Stanek K, Drobne D and Trebse P, 2006. Linkage of biomarkers along levels of biological complexity in juvenile and adult diazinon fed terrestrial isopod (*Porcellio scaber*, Isopoda, Crustacea). Chemosphere, 64, 1745–1752.
Stewart A, Brownbridge M, Hill R and Jackson T, 2010. Utilizing Soil Microbes for Biocontrol. In: *Soil Microbiology and Sustainable Crop Production*. Eds Dixon R, Tilston E, Springer, 315–371.
Sturm CF, Pearce TA and Valdes A, 2006. The mollusks: a guide to their study, collection, and preservation. Eds Sturm CF, Pearce TA, Valdes A. Universal Publishers, 457 pp.
Suter GW, 1993. *Ecological risk assessment*. Second Edition, CRC Press, 680 pp.
Sverdrup LE, De Vaufleury A, Hartnik T, Hagen SB, Loibner AP and Jensen J, 2006. Effects and uptake of polycyclic aromatic compounds in snails (Helix aspersa). Environmental Toxicology and Chemistry, 25, 1941–1945.
Swift MJ, Heal OW and Anderson JM, 1979. Decomposition in terrestrial ecosystems. Eds Swift MJ, Heal OW, Anderson JM. University of California Press, Berkeley, 372 pp.
Swift MJ, Izac AMN and van Noordwijk M, 2004. Biodiversity and ecosystem services in agricultural landscapes – are we asking the right questions? Agriculture Ecosystems & Environment, 104, 113–134.
Szukics U, Abell G, Hoedl V, Mitter B, Sessitsch A, Hackl E and Zechmeister-Boltenstern S, 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. Fems Microbiology Ecology, 72, 395–406.
Tempesta T, 2010. The perception of agrarian historical landscapes: a study of the Veneto plain in Italy. Landscape and Urban Planning, 97, 258–272.
Termorshuizen A and Jeger M, 2008. Strategies of soilborne plant pathogenic fungi in relation to disease suppression. Fungal Ecology, 1, 108–114.
Teuben A, 1991. Nutrient availability and interactions between soil arthropods and microorganisms during decomposition of coniferous litter-a mesocosm study. Biology and Fertility of Soils, 10, 256–266.
Teuben A and Roelofsma T, 1990. Dynamic interactions between functional groups of soil arthropods and microorganisms during decomposition of coniferous litter in microcosm experiments. Biology and Fertility of Soils, 9, 145–151.
Timper P, Davis R, Jagdale G and Herbert J, 2012. Resiliency of a nematode community and suppressive service to tillage and nematicide application. Applied Soil Ecology, 59, 48–59.
Topping C and Odderskaer P, 2004. Modeling the influence of temporal and spatial factors on the assessment of impacts of pesticides on skylarks. Environmental Toxicology and Chemistry, 23, 509–520.
Topping CJ, Kjaer L, Hommen U, Hoye T, Preuss T, Sibly R and van Vliet P, 2014. Recovery based on plot experiments is a poor predictor of landscape-level population impacts of agricultural pesticides. Environmental Toxicology and Chemistry, 33, 1499–1507.
Topping C, Craig P, de Jong F, Klein M, Laskowski R, Manachini B, Pieper S, Smith R, Sousa J, Streissl F, Swarowsky K, Tiktak A and van der Linden T, 2015a. Towards a landscape scale management of pesticides: ERA using changes in modelled occupancy and abundance to assess long-term population impacts of pesticides. Science of the Total Environment, 537, 159–169.
Topping CJ, Alrøe HF, Farrell KN and Grimm V, 2015b. Per Aspera ad Astra: through Complex Population Modeling to Predictive Theory. The American Naturalist, 186, 669–674.
Topping CJ, Dalby L and Skov F, 2016. Landscape structure and management alter the outcome of a pesticide ERA: evaluating impacts of endocrine disruption using the ALMaSS European Brown Hare model. Science of the Total Environment, 541, 1477–1488.
Torsvik V and Ovreas L, 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology, 5, 240–245.

Toschki A, Hommen U, Klein K, Koenig W, Pieper S, Römbke R, Ross-Nickoll M, Schäffer A, Possberg C, Scholz-Starke B and Hammers-Wirtz M, 2014. Evaluation of the risk for soil organisms under real conditions. Proceedings of the SETAC Europe 24th Annual meeting, Basel, Switzerland.

Toschki A, Hammers-Wirtz M, Poßberg C, Roß-Nickoll M, Schäffer A, Schmidt B, Scholz Starke B, Römbke J, Scheffzyk A, Klein M and Hommen U, 2015. Evaluation of the risk for soil organisms under real conditions – Development of a national position in the context of the new European Plant Protection Regulation (EU 1107/2009). Federal Environment Agency Umweltbundesamt FKZ 3710 67 410 Final report.

Traas TP, Janse JH, van den Brink PJ, Brock TCM and Aldenberg T, 2004. A freshwater food-web model for the combined effects of nutrients and insecticide stress and subsequent recovery. Environmental Toxicology and Chemistry, 23, 521–529.

Trichard A, Alignier A, Biju-Duval L and Petit S, 2013. The relative effects of local management and landscape context on weed seed predation and carabid functional groups. Basic and Applied Ecology, 14, 235–245.

Triebskorn R, 2005. Biomarkers, bioindicators and the Trondheim Biomonitoring System. In: Lehr JH, Keeley J (eds.). *Water Encyclopedia: Water Quality and Resource Development*. John Wiley and Sons, New York, USA. pp. 29–33.

Triebskorn R, 2009. Schnecken unter Stress. Gastropoden als Modelle in Ökophysiologie und Ökotoxikologie. Umweltwiss Schadst Forsch 21: 150-159.Triebskorn R, Bader K, Linder B and Koehler H-R, 2005. Snails and slugs as non-targets for environmental chemicals. IOBC-WPRS Bulletin, 28, 1–7.

Tsiafouli MA, Thebault E, Sgardelis SP, de Ruiter PC, van der Putten WH, Birkhofer K, Hemerik L, de Vries FT, Bardgett RD, Brady MV, Bjornlund L, Jorgensen HB, Christensen S, Hertefeldt T, Hotes S, Hol WHG, Frouz J, Liiri M, Mortimer SR, Setala H, Tzanopoulos J, Uteseny K, Pizl V, Stary J, Wolters V and Hedlund K, 2015. Intensive agriculture reduces soil biodiversity across Europe. Global Change Biology, 21, 973–985.

Tuomisto H, Hodge I, Riordan P and Macdonald D, 2012. Does organic farming reduce environmental impacts? A meta-analysis of European research. Journal of Environmental Managenment, 112, 309–320.

Turbé A, De Toni A, Benito P, Lavelle P, Ruiz N, Van der Putten W, Labouze E and Shailendra M, 2010. Soil biodiversity: functions, threats and tools for policy makers. Bio Intelligence Service, IRD and NIOO. Report for the European Commission (DG Environment).

Turrini A and Giovannetti M, 2012. Arbuscular mycorrhizal fungi in national parks, nature reserves and protected areas worldwide: a strategic perspective for their *in situ* conservation. Mycorrhiza, 22, 81–97.

UBA (Umweltbundesamt), 1994. UBA-workshop on terrestrial model ecosystem.

Umer M and Rajab S, 2012. Correlation between aggregate stability and microbiological activity in two Russian soil types. Eurasian Journal of soil science, 1, 45–50.

Umweltbundesam, 2011. Exposit 3.0 beta version. Available online: www.bvl.bund.de/DE/04_Pflanzenschutzmittel/03_Antragsteller/04_Zulassungsverfahren/07_Naturhaushalt/psm_naturhaush_node.html

USEPA (US-Environmental Protection Agency), 1996. Ecological effects test guideline. OPPTS 850.2450. Terrestrial (soil-core) microcosm test. Public draft.

USEPA (US Environmental Protection Agency), 1998. Guidelines for ecological risk assessment. Washington (DC): USEPA Risk Assessment Forum. EPA/630/R-95/002F.

USEPA (US-Environmental Protection Agency), 1999. Use of monitored natural attenuation at Superfund, RCRA corrective action, and underground storage tank sites. 41 p.

USEPA (US Environmental Protection Agency), 2004. Generic ecological assessment endpoints (GEAEs). Washington (DC): USEPA Risk Assessment Forum. EPA/630/P-02/004F.

Usher MB, 1970. Seasonal and vertical distribution of a population of soil arthropods: Collembola. Pedobiologia, 10, 224–236.

Van Brummelen T, Verweij R, Wedzinga S and VanGestel C, 1996. Polycyclic aromatic hydrocarbons in earthworms and isopods from contaminated forest soils. Chemosphere, 32, 315–341.

Van de Zande J, Rautmann D, Holterman H and Huijsmans J, 2015. Joined spray drift curves for boom sprayers in The Netherlands and Germany. 82 pp.

Van der Heijden MGA, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A and Sanders IR, 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytologist, 172, 739–752.

Van der Heijden MGA, Bardgett RD and van Straalen NM, 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11, 296–310.

Van der Linden A, Boesten JJTI, Brock T, Van Eekelen G, De Jong F, Leistra M, Montforts M and Pol J, 2006. Persistence of plant protection products in soil; a proposal for risk assessment. RIVM, Bilthoven, Report 601506008.

Van der Linden A, Boesten J, Brock T, Van Eekelen G, Ter Horst M, De Jong F, Montforts M and Pol J, 2008. Revised proposal for the risk assessment of persistence of plant protection products in soil. RIVM report 601712003.

Van Eekeren N, Bommele L, Bloem J, Schouten T, Rutgers M, de Goede R, Reheul D and Brussaard L, 2008. Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. Applied Soil Ecology, 40, 432–446.

Van Gestel CAM, 1992. The influence of soil characteristics on the toxicity of chemicals for earthworms: a review. In: *Ecotoxicology of Earthworms*. Eds Greig-Smith PW, Becker H, Edwards PJ, Heimbach F. Cambridge University Press, 390 pp.
Van Gestel CAM, 1997. Scientific basis for extrapolating results from soil ecotoxicity tests to field conditions and the use of bioassays. Eds vanStraalen NM and Lokke H.
Van Gestel CAM, 2012. *Soil ecotoxicology: state of the art and future directions*. Zookeys, 275-296.
Van Gestel CAM and Ma W, 1988. Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil. Ecotoxicology and Environmental Safety, 15, 289–297.
Van Gestel CAM and Ma W, 1990. An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere, 21, 1023–1033.
Van Straalen NM and Løkke H, 1997. *Ecological Risk Assessment of Contaminants in Soil*. Springer Science & Business Media, 333 pp.
Van Vliet P, Radcliffe D, Hendrix P and Coleman D, 1998. Hydraulic conductivity and pore-size distribution in small microcosms with and without Enchytraeids (Oligochaeta). Applied Soil Ecology, 9, 277–282.
Van Wensem J and Adema T, 1991. Effects of fluoride on soil mediated litter decomposition. Environmental Pollution, 72, 239–251.
Van Wensem J, Jagers Op Akkerhuis G and Van Straalen N, 1991. Effects of the fungicide Triphenyltin hydroxide on soil fauna mediated litter decomposition. Pesticide Science, 32, 307–316.
Van Wensem J, Jagers Op, Akkerhuis G and Van Straalen N, 1992. Effects of the fungicide fentin hydroxide on isopod interaction with mineralization processes. Proceedings of the international symposium on environmental aspects of pesticides microbiology. Eds Anderson JP. Symposium Uppsala, Sweden, 440 pp.
Van Wensem J, VanStraalen NM and Kooijman S, 1997. Carbon and nitrogen fluxes in decomposing leaf litter with microbial-detritivore interactions: model simulations compared to microcosm ecotoxicity tests. Ecological Modelling, 96, 175–189.
Vanbergen AJ, Watt AD, Mitchell R, Truscott A-M, Palmer SCF, Ivits E, Eggleton P, Jones TH and Sousa JP, 2007. Scale-specific correlations between habitat heterogeneity and soil fauna diversity along a landscape structure gradient. Oecologia, 153, 713–725.
Vanderwalle M, Sykes M, Harrison P, Luck G, Berry P, Bugter R, Dawson T, Feld C, Harrington R, Haslett J, Hering D, Jones K, Jongman R, Lavorel S, Martins da Silva P, Moora M, Paterson J, Rounsevell M, Sandin L, Settele J, Sousa J and Zobel M, 2008. Review paper 2235 on concepts of dynamic ecosystems and their services. Rubicode Project.
Venter J and Reinecke A, 1988. The life-cycle of the compost worm *Eisenia fetida* (Oligochaeta). South African Journal of Zoology, 23, 161–165.
Verhoef H, 1984. Releaser and primer pheromones in Collembola. Journal of Insect Physiology, 30, 665–670.
Verhoef H and Brussaard L, 1990. Decomposition and nitrogen mineralization in natural and agroecosystems-The contribution of soil animals. Biogeochemistry, 11, 175–211.
Verhoef H and Nagelkerke C, 1977. Formation and ecological significance of aggregations in Collembola-Experimental study. Oecologia, 31, 215–226.
Verro R, Finizio A, Otto S and Vighi M, 2009. Predicting pesticide environmental risk in intensive agricultural areas.II. Screening level risk assessment of complex mixtures in surface waters. Environmental Science andTechnology, 43, 522–529.
Viard B, Maul A and Pihan JC, 2004. Standard use conditions of terrestrial gastropods in active biomonitoring of soil contamination. Journal of Environmental Monitoring, 6, 103–107.
Vink K and Van Straalen NM, 1999. Effects of benomyl and diazinon on isopod-mediated leaf litter decomposition in microcosms. Pedobiologia, 43, 345–359.
Vink K, Tompot A, Hermans M, van Straalen NM, Dewi I and Bedaux J, 1995. The importance of the exposure route when testing the toxicity of pesticides to saprotrophic isopods. Environmental Toxicology and Chemistry, 14, 1225–1232.
Viswanathan R, 1997. Physiological basis in the assessment of ecotoxicity of pesticides to soil organisms. Chemosphere, 35, 323–334.
Vollmer T, Klein O, Frank S and Knaebe S, 2016. *Statistical Power and MDDs in Earthworm field testing*. Poster Eurofins.
Wagner M, 2004. Deciphering functions of uncultured microorganisms. Asm News, 70, 63–70.
Wall DH and Moore JC, 1999. Interactions underground - Soil biodiversity, mutualism, and ecosystem processes. BioScience, 49, 109–117.
Wall DH, Bardgett RD, Covich AR and Snelgrove PVR, 2004. The need for understanding how biodiversity and ecosystem functioning affect ecosystem services in soils and sediments. In: Wall DH (ed.). *Sustaining biodiversity and ecosystem services in soils and sediments*. Press, Washington DC, Island. pp. 1–12.
Wallwork J, 1970. *The Ecology of Soil Animals*. McGraw Hill, London.
Wang M and Grimm V, 2010. Population models in pesticide risk assessment: lessons for assessing population-level effects, recovery, and alternative exposure scenarios from modelling a small mammal. Environmental Toxicology and Chemistry, 29, 1292–1300.
Warburg M, 1993. *Evolutionary biology of land isopods*. Springer-Verlag, Berlin, Heidelberg 159 pp.

Warburg M, Calahorra Y and Amar K, 1991. Non-seasonal breeding in a porcellionid isopod. Journal of Crustacean Biology, 21, 375–383.
Wartiainen I, Eriksson T, Zheng W and Rasmussen U, 2008. Variation in the active diazotrophic community in rice paddy - nifH PCR-DGGE analysis of rhizosphere and bulk soil. Applied Soil Ecology, 39, 65–75.
Weber J, 2007. Accounting for soil in the SEEA. European Environment Agency, Rome. Available at: http://mdgs.un.org/unsd/envaccounting/londongroup/meeting12/LG12_9a.pdf
Weigmann G, 1998. Bodenfauna. In: *Handbuch der Bodenkunde*. Eds Blume H, Felix-Henningsen P, Fischer W, Frede H-G, Horn R, Stahr K, Ecomed, Landsberg.
Weinstoerffer J and Girardin P, 2000. Assessment of the contribution of land use pattern and intensity to landscape quality: use of a landscape indicator. Ecological Modelling, 130, 95–109.
Weissenfels W, Beyer M and Klein J, 1992. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Applied Microbiology and Biotechnology, 35, 689–696.
Weller D, Raaijmakers J, Gardener B and Thomashow L, 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology, 40, 309–348.
Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, Guillaumaud N and Le Roux X, 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environmental Microbiology, 9, 2211–2219.
Westheide W and Graefe U, 1992. Two new terrestrial *Enchytraeus* species (Oligochaeta, Annelida). Journal of Natural History, 26, 479–488.
Weyland F and Laterra P, 2014. Recreation potential assessment at large spatial scales: a method based in the ecosystem services approach and landscape metrics. Ecological Indicators, 39, 34–43.
Whipps J and Gerhardson B, 2007. Biological pesticides for control of seed- and soil-borne plant pathogens. In: *Modern Soil Microbiology*. 2nd Edition. Ed Van Elsas JD JJ, Trevors JT, CRC Press, FL, 479–501.
Whitford WG, Freckman DW, Elkins NZ, Parker LW, Parmalee R, Phillips J and Tucker S, 1980. Diurnal migration and responses to simulated rainfall in desert soil microarthropods and nematodes. Soil Biology & Biochemistry, 13, 417–425.
WHO/IPCS, 2014. Guidance document on evaluating and expressing uncertainty in hazard 6440 characterization. Harmonization Project Document No. 11. International Programme on 6441 Chemical Safety. WHO, Geneva.
Wieser W, 1978. A comparative analysis of soil fauna populations and their role in decomposition processes. Oecologia, 36, 191–201.
van Wijnen H, Rutgers M, Schouten A, Mulder C, de Zwart D and Breure A, 2012. How to calculate the spatial distribution of ecosystem services - Natural attenuation as example from The Netherlands. Science of the Total Environment, 415, 49–55.
Wilby A and Thomas M, 2002. Natural enemy diversity and pest control: patterns of pest emergence with agricultural intensification. Ecology Letters, 5, 353–360.
Wiles JA and Frampton GK, 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. Pesticide Science, 47, 273–285.
Wilson M and Jackson T, 2013. Progress in the commercialisation of bionematicides. BioControl, 58, 715–722.
Wilson GWT, Rice CW, Rillig MC, Springer A and Hartnett DC, 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters, 12, 452–461.
Wolfarth F, Schrader S, Oldenburg E and Weinert J, 2011a. Earthworms promote the degradation of Fusarium biomass and deoxinivalenol content in wheat straw under field conditions. Soil Biology and Biochemistry, 43, 1858–1865.
Wolfarth F, Schrader S, Oldenburg E and Weinert J, 2011b. Contribution of the endogeic earthworm species *Aporrectodea caliginosa* to the degradation of deoxynivalenol and Fusarium biomass in wheat straw. Mycotoxin Research, 27, 215–222.
Wolfarth F, Schrader S, Oldenburg E and Weinert J, 2013. Nematode-collembolan-interaction promotes the degradation of Fusarium biomass and deoxynivalenol according to soil texture. Soil Biology and Biochemistry, 57, 903–910.
Wolfarth F, Schrader S, Oldenburg E and Brunotte J, 2015. Regulation of the mycotoxin deoxynivalenol by *Folsomia candida* (Collembola) and *Aphelenchoides saprophilus* (Nematoda) in an on-farm experiment. Pedobiologia, 58, 41–47.
Wolters V, 2001. Biodiversity of soil animals and its function. European Journal of Soil Biology, 37, 221–227.
Wolters V and Ekschmitt K, 1997. Gastropods, Isopods, Diplopods, and Chilopods: neglected Groups of the Decomposer Food Web, in: *Fauna in Soil Ecosystems: recycling Processes, Nutrient Fluxes, and Agricultural Production, Chapter: 8*., Publisher: Marcel Dekker Inc (New York), Editors: Gero Benckiser, pp. 265–306.
Wright S and Upadhyaya A, 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil, 198, 97–107.
Yachi S and Loreau M, 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proceedings of the National Academy of Sciences of the United States of America, 96, 1463–1468.
Yeates G, 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils, 37, 199–210.

Yeates GW, Dando JL and Shepherd TG, 2002. Pressure plate studies to determine how moisture affects access of bacterial-feeding nematodes to food in soil. European Journal of Soil Science, 53, 355–365.
Yu YL, Shan M, Fang H, Wang X and Chu XQ, 2006. Responses of soil microorganisms and enzymes to repeated applications of chlorothalonil. Journal of Agricultural and Food Chemistry, 54, 10070–10075.
van Zanten B, Verburg P, Espinosa M, Gomez-y-Paloma S, Galimberti G, Kantelhardt J, Kapfer M, Lefebvre M, Manrique R, Piorr A, Raggi M, Schaller L, Targetti S, Zasada I and Viaggi D, 2013. European agricultural landscapes, common agricultural policy and ecosystem services: a review. Agronomy for Sustainable Development, 34, 309–325.
Zasada IA, Elmore CL, Yakabe LE and Macdonald JD, 2007. Evaluation of Propargyl Bromide as a Soil Fumigant. HortScience, 42, 1212–1216.
Zhang F-G and Zhang Q-G, 2015. Patterns in Species Persistence and Biomass Production in Soil Microcosms Recovering from a Disturbance Reject a Neutral Hypothesis for Bacterial Community Assembly. PLoS ONE, 10.
Zhang J, Liang W, Wu X, Jiang S and Li Q, 2013. Toxic effects of acetochlor on mortality, reproduction and growth of *Caenorhabditis elegans* and *Pristionchus pacificus*. Bulletin of environmental contamination and toxicology, 90, 364–368.
Zhao J and Neher D, 2013. Soil nematode genera that predict specific types of disturbance. Applied Soil Ecology, 64, 135–141.
Zidar P, Hribar M, Zizek S and Strus J, 2012. Behavioural response of terrestrial isopods (Crustacea: Isopoda) to pyrethrins in soil or food. European Journal of Soil Biology, 51, 51–55.

Glossary and Abbreviations

a.s.	active substance
ABM	agent-based modelling
AF	assessment factor
ASTM	American Society for Testing and Materials
BAF	bioaccumulation factor
BSAF	biota to soil accumulation factor
CRP	Community Recovery Principle
$DegT_{50}$	half-life in a medium due to degradation (transformation) processes
DT_{50}	half-life in a medium due to degradation (transformation) and other processes such as volatilisation and leaching
EC_x	concentration at which x% effect was observed/calculated
EPPO	European and Mediterranean Plant Protection Organization
EPN	entomopathogenic nematodes
ERA	environmental risk assessment
ERC	ecotoxicologically relevant concentration
ES	ecosystem service
ETP	ecological threshold principle
FOCUS	FOrum for Co-ordination of pesticide fate models and their USe
FRP	Functional Redundancy Principle
GAP	good agricultural practice
HC	hazardous concentration
HSP	heat shock protein
IBM	individual-based modelling
IOBC	International Organization of Biological Control
IP	integrated production
IPM	Integrated Pest Management
ISO	International Organization for Standardization
K_{OM}	Coefficient of equilibrium sorption on organic matter (L/kg)
LC_{50}	lethal concentration, median
Log K_{OW}	partition coefficient *n*-octanol/water
MDD	minimal detectable difference
Metabolite	Any metabolite or a degradation product of an active substance, safener or synergist, formed either in organisms or in the environment (thus including also oxidation products which may have a larger molecular mass than the parent substance).
m_{om}	mass fraction of organic matter in the soil (in kg/kg)
M_{soil}	total mass of soil in the system (in kg)
M_t	total amount of substance applied (in kg)
MLO	mycoplasma-like organisms

NEL	no effect level
NOEC	no observed effect concentration
NOR	normal operating range
NTA	non-target arthropod
NTTP	non-target terrestrial plant
OECD	Organisation for Economic Cooperation and Development
PAH	polycyclic aromatic hydrocarbons
PBT	Persistent, Bioaccumulating and Toxic
PCR	polymerase chain reaction
PEARL	Pesticide Emission At Regional and Local Scales
PEC	predicted environmental concentration
PELMO	Pesticide Leaching Model
PERSAM	Persistence in Soil Analytical Model) and new versions of the pesticide fate models
PLFA	phospholipid fatty acid analysis
PPPs	plant protection products
PPR Panel	EFSA's Scientific Panel on Plant Protection Products and their Residues
POP	persistent organic pollutant
QSAR	quantitative structure–activity relationships
RA	risk assessment
RAC	regulatory acceptable concentraion
REG	regulation
RMS	rapporteur Member State
S_f	scaling factor
SPGs	specific protection goals
SPU	Service Providing Units
SRT	surrogate reference tier
SSD	Species Sensitivity Distribution
TER	toxicity exposure ratio (i.e. NOEC/PEC or EC_{10}/PEC)
TK/TD	toxicodynamics/toxicokinetic
TME	terrestrial model ecosystem
Vital rate	the relative frequency of vital occurrences that affect changes in the size and composition of a population, i.e. the rates of births and deaths.
vPvT	very Persistent, very Toxic
V_{soil}	total volume of liquid in the system (in L)

Appendix A – Information on the biology of in-soil organisms in the scope of this Opinion

Macrofauna are relatively large organisms (see Figure 1) and include decomposers, herbivores, predators and the so-called 'ecosystem engineers'. Most insects, spiders, isopods, myriapods and others belong to the 'macroarthropods'. Other important macrofauna include soft-bodied, legless soil biota such as annelids and gastropods.

'Ecosystem engineers' have been defined as 'organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they modify, maintain and create habitats' (Jones et al., 1996). Among soil fauna, earthworms, termites and ants have been identified as the most important soil engineers. Termites and ants are mentioned for completeness, even though these two groups are not covered in this Opinion. Termites mostly occur in the tropics, with only few species known from natural habitats in Europe, none being associated with agricultural fields. Termites are social insects organised into castes. They have the ability to digest wood and other lignocellulosic substrates thanks to their intestinal microbiota (Dietrich et al., 2014). Termites are classified according to their feeding behaviour in different groups, e.g. wood-feeders, soil-feeders or humivores, fungus feeders, etc. (Donovan et al., 2001). Ants, together with other Hymenoptera, are covered by the scientism opinion on non-target arthropods (EFSA PPR Panel, 2015). They are social insects that often nest in soil and consume a variety of foods, depending upon species such as microarthropods, decaying organic debris, seeds, plant secretions, and aphid secretions. Ants forming surface mounds are important in mixing soil from lower depths with surface soil. Therefore, they could modify soil chemical and physical properties by transporting food and soil materials during feeding, mound and gallery construction. Ants can affect plant productivity as they can have different types of relationship with plants (Gonzalez-Teuber et al., 2014).

Earthworms are well-known because of their ability to consume, excrete and organise mineral and organic constituents of soil. *Eisenia fetida* and *E. andrei* are popular commercial earthworms living in soil rich in organic matter, although they are not typical soil species, and their biology is relatively well known (OECD 207; McElroy and Diehl, 2001). *E. fetida* and *E. andrei* are similar from a morphological point of view, although they have a different pigmentation (striped morph and uniformly reddish morph, respectively). Two other well-studied species are *Lumbricus terrestris*, which burrows vertically deep into the soil, and *Aporrectodea caliginosa* (formerly *Allolobophora caliginosa)*, which dwells mainly at the topsoil layer. Burrows loosen soil, allowing roots and other soil animals to colonise this space. Earthworm casts are often enriched in organic matter, microbial populations, and nutrient content, which improves nutrient cycling.

Isopods are cryptozoans, i.e. surface soil dwellers under stones, bark, or litter layers, which emerge at night to forage and might show the ability of rolling into balls as a defence mechanisms and to avoid desiccation. They feed on roots, vegetation, and decaying plant litter, resulting in considerable fragmentation of organic matter.

Millipedes are saprophagous feeders, i.e. consuming dead or decaying organic debris, with a calcareous exoskeleton, and therefore important in calcium cycling.

Centipedes are elongate, flattened, and active predators of various microarthropods in soil and surface litter.

Terrestrial gastropods are often recognised as agricultural pests. However, only a relatively small fraction of species are considered as pest or parasite vector, whereas the greater part of terrestrial gastropod diversity are very small animals living as detrivores in the litter layer (Barker, 2001). There are several studies showing the occurrence of non-target gastropod species in the agricultural landscape at cropped sites and adjacent habitats. Different studies in Europe indicated the occurrence of non-target gastropod species in the agricultural landscape (fields and field margins of different crops), see e.g. Biodiversitätsmonitoring Schweiz BDM (2014), Willecke (1983) and Swarowsky et al. (2013) at relatively high abundances. There is an approaching crisis for the conservation of non-marine molluscs, which account for approximately 40% of the known animal extinctions in the last 400 years (Bouchet et al. 1999), Looking at the red list of endangered species, it can be seen that a large proportion of open-land species that may inhabit different habitats in the agricultural landscape can be considered to be at risk. For example, 76% of the German open-land snail species are at least weakly endangered (Jungbluth and Knorre 2009).

Some terrestrial organisms living in and on agricultural soil might spend only part of the life cycle in the soil. The following groups are considered to be non-target arthropods, even though they have life stages present in the soil matrix. Spiders are typically predators of insects in soil and surface-litter

layers. Solitary wasps construct nests in soil and prey on other insects or spiders to feed developing eggs. Beetles are the most diverse insect family, and in the soil can be divided into predacious, leaf feeding, and saprophagous. Ground beetles, rove beetles, and tiger beetles are predators of other insects and are important pest-control agents in agroecosystems. Dung beetles feed typically on large-animal faeces. Wireworms are beetle larvae that feed on roots (Lavelle, 1996; Lavelle and Spain, 2005; Wall et al., 2012).

Mesofauna include mainly Acari, Collembola, Enchytraeidae, Tardigrada, Protura and Diplura. Acari, or mites, are often the most abundant and the most species-rich group of the soil mesofauna. Mites inhabit air-filled soil pores and litter and have a hard body. Soil mites are a very diverse assemblage of Arachnida, divided into four major groups: oribatids, prostigmatics, mesostigmatics and astigmatics. Oribatid mites are morphologically distinct between juvenile and adult stages, their reproduction is generally slow and typically feed on detritus and fungi. Oribatid mites have a calcareous exoskeleton. Prostigmatic mites feed on fungi, algae and other soil organisms. Mesostigmatic mites are mostly predators of nematodes and microarthropods. Although Oribatid mites are usually considered the most abundant mites in soils and Astigmatic mites the least common it is also reported that astigmatic mites may become dominant in some habitat (Coleman et al. 2004).

Collembola (or springtails) are small (0.2 to 5 mm in length), primitive hexapods. The number of Collembola species known is much lower than that of Acari, but they can reach the same abundances. Like Acari, Collembola live in air-filled soil pores and litter. Most Collembola eat decaying vegetation and fungi, although they have also been observed to consume nematodes and plant roots throughout the soil profile. Collembola are opportunistic microarthropods, capable of rapid individual and population growth when conditions are favourable. They may be important biological control agents for crops by consuming pathogenic fungi. Eggs are laid in groups and, therefore, populations occur in aggregations rather than at random. Collembola can be important food sources for predacious mites, beetles, and ants.

Enchytraeidae (potworms) are similar to small earthworms, also belonging to Oligochaeta. They are most abundant in wet, organic soils and feed on detritus, algae, bacteria, fungi and other soil organisms. They live in the litter and in the upper few centimetres of soil because of their limited ability to move long distances. Some species can burrow in the sand while others can move vertically using earthworm casts (Lavelle and Spain, 2005).

Other mesofauna that are less numerous, but present in many soils, are Tardigrada (soft, plump body and eight poorly articulated limbs with claws), Protura (wingless hexapods lacking antennae and eyes that live near plant roots and litter), Diplura (elongate, delicate hexapods with long antennae and two abdominal cerci that feed either on decaying vegetation or predacious on nematodes, springtails, and potworms), pseudoscorpions (small arachnids that feed on nematodes, microarthropods, and potworms), symphylids (white, eyeless, elongate, many-legged invertebrates resembling centipedes that feed on vegetation and soft soil animals) and Pauropoda (another group of centipede-like, colourless arthropods with branched antennae, that feed on fungi and other soil organisms).

Microfauna are represented mostly by rotifers and nematodes. Rotifers can be found in soils that are continually moist, but are typically aquatic organisms and are therefore not considered important in-soil organisms.

Nematodes (also called roundworms, threadworms, or eelworms) are among the most abundant metazoan organisms in soil and are important components of the soil food web. Nematodes vary widely in their feeding strategies, being bacterivores, fungivores, plant feeders, pathogens of vertebrates and invertebrates, carnivores or omnivores. Nematodes proliferate in the water-filled pores of soil or plant roots. They have low motility, and they are often susceptible to stressors. Most nematodes are saprophytic, i.e. feeding on decaying organic matter. Nematodes are considered to be potentially holistic indicators of soil processes as they are active within the soil throughout the year. In addition, some species may be important biological control agents for crops by consuming pathogenic fungi and bacteria and being entomopathogenic agents (controlling some insect pests, and also molluscs). In agroecosystems, various nematode groups play critical roles in plant productivity. Bacterial- and fungal-feeding nematodes contribute to decomposition of organic matter and the release of nutrients for plant uptake (Ferris et al., 2004). Plant-feeding nematodes, however, can impair root function and act as nutrient sinks, thereby reducing crop yield (Luc et al., 2005). Predatory nematodes and some omnivorous nematodes prey on small invertebrates, including plant-feeding nematodes and may regulate populations of these pests (McSorley et al., 2008; Khan and Kim, 2007). Specific indices have been designed to assess soil quality using nematode communities (Yeates and Williams, 2001; Griffiths et al., 2002; Okada and Harada, 2007; Manachini et al., 2009). These indices

are based on the concept that particular taxa differ in sensitivity to stressors or disruptions of the successional sequence because of their life history characteristics. Nematode taxa were rated along a coloniser–persister (c–p) scale of 1–5 according to Bongers and Ferris (1999), roughly equivalent to the range from extreme r- to extreme K-strategists. 'Coloniser' nematodes at the lower end of the c-p scale are considered enrichment opportunists and therefore indicate resource availability; 'persister' nematodes at the high end of the scale indicate system stability, high food web complexity and high connectance. In addition to these, the metabolic footprint could also be estimated using nematodes. It is an estimator of nematode contribution to various ecosystem services and functions. Nematodes are considered as potential bioindicators in assessing the impact of different toxicants, including plant protection products (PPPs), and other potential stressors on soil ecosystems and in general of soil health. They have been shown to be sensitive enough to indicate effects after exposure to relatively low concentrations of toxic chemicals (Kammenga et al., 2000; Nagy, 2009; Shashikumar and Rajini, 2010; Höss et al., 2014).

In this scientific opinion, **microorganisms** (or 'microbes') collectively comprise single-celled organisms with heterotrophic organotrophic (saprophytes) or chemolithotrophic autotrophic lifestyle. Photosynthetic autotrophic microorganisms, i.e. cyanobacteria and eukaryotic algae can be also present in surface soil layers, but they play a smaller role in the majority of agricultural soils (although the N-fixing cyanobacteria can be important, for example, in rice plantations). Protozoans are eukaryotic, single-celled, non-photosynthetic organisms that are not fungi. The group is polyphyletic and, based on morphology, most protozoans are ciliates, flagellates or amoeboids. Unlike the bacteria and archaeans (lacking nucleus and other organelles and collectively referred to as prokaryotes) and fungi in which osmotrophic nutrition dominates, most protozoans are phagotrophic and ingest particulate matter (preferably bacteria) (Epstein, 1997). Not all microorganisms in soil occur as free-living organisms in pores of various dimensions, but many live in symbiosis with other organisms. For instance, specific microbial communities are associated with the gut system of soil-dwelling insect larvae (e.g. Andert et al., 2010) and collembolans (Thimm et al., 1998). Another example is the plant rhizosphere, which is characterised by high metabolic activity and harbours very diverse microbial communities (Van der Heijden et al., 2006; Weinert et al., 2011; Kuzyakov and Blagodatskaya, 2015). Among microorganisms forming symbiotic interactions, mycorrhizal fungi have been identified in thousands of plant species among all major plant lineages including bryophytes, ferns, gymnosperms and angiosperms (Wang and Qiu, 2006; Brundrett, 2009). Six different types of mycorrhizal symbiosis have been identified (Kernaghan, 2005), although the most abundant and well-studied are the arbuscular mycorrhizal fungi (AMF), the ectomycorrhizal fungi (EM) and the ericoid mycorrhizae (ERM) (Van der Heijden et al., 2008). The arbuscular mycorrhizae are abundant in grassland, savannah and tropical forests, they also associate with many grasses and are able to establish interaction with roots of 80% of plant families (Gianinazzi et al., 2010). Ectomycorrhizal (EM) fungi are widespread in temperate and boreal forest and comprise over 20,000 species forming root symbiotic with many long-lived perennial plants and trees (Finlay, 2008). Ectomycorrhizae are predominantly from the Basidiomycota and Ascomycota. Ericoid mycorrhiza are formed in three plant families, the Ericaceae, Empetraceae and Epacridaceae, all belonging to the order Ericales (Finlay, 2008). Due to the low relevance of this group of mycorrhizae for crop plants, they are not further addressed in this Opinion. Bacteria belonging to the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium* and *Azorhizobium* (collectively referred to as rhizobia) grow in the soil as free-living organisms but can also live as nitrogen-fixing symbionts inside root-nodule cells of legume plants. The fundamental life-supporting roles that microorganisms have in nature are now considered an obvious component of ecosystem-services frameworks (Ducklow, 2008; EFSA PPR Panel, 2010a; Bodelier, 2011), and microbes are critical components in the discussion on soil health and ecosystem services of arable soils (Barrios, 2007; Kibblewhite et al., 2008).

References

Andert J, Marten A, Brandl R and Brune A, 2010. Inter- and intraspecific comparison of the bacterial assemblages in the hindgut of humivorous scarab beetle larvae (Pachnoda spp.). Fems Microbiology Ecology, 74, 439–449.

Barker G, 2001. The biology of terrestrial molluscs. Oxon, UK: CABI Publishing, 558 pp.

Barrios E, 2007. Soil biota, ecosystem services and land productivity. Ecological Economics, 64, 269–285.

Bodelier PLE, 2011. Toward understanding, managing, and protecting microbial ecosystems. Frontiers in Microbiology, 2, 1–8.

Bongers T and Ferris H, 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution, 14, 224–228.

Bouchet P, Falkner G, Seddon MB. 1999. Lists of protected land and freshwater molluscs in the Bern Convention and European Habitats Directive: are they relevant to conservation? Biological Conservation 90: 21–31.
Brundrett MC, 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil, 320, 37–77.
Coleman DC, Crossley DA, Hendrix PF, 2004. Fundamentals of soil ecology, 2nd Edition. Eds Coleman DC, Crossley DA, Hendrix PF. Elsevier Academic Press, 386 pp.
Dietrich C, Köhler T and Brune A, 2014. The Cockroach Origin of the Termite Gut Microbiota: Patterns in Bacterial Community Structure Reflect Major Evolutionary Events. Applied and Environmental Microbiology, 80, 2261–2269.
Donovan S, Eggleton P and Bignell D, 2001. Gut content analysis and a new feeding group classification of termites. Ecological Entomology, 26, 356–366.
Ducklow H, 2008. Microbial services: challenges for microbial ecologists in a changing world. Aquatic Microbial Ecology, 53, 13–19.
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010. Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal, 8, 1821,1855 pp.
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal, 13, 3996, 3212 pp.
Epstein SS, 1997. Microbial food webs in marine sediments 1. Trophic interactions and grazing rates in two tidal flat communities. Microbial Ecology, 34, 188–198.
Ferris H, Venette RC and Scow KM, 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Applied Soil Ecology, 25, 19–35.
Finlay RD, 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. Journal of Experimental Botany, 59, 1115–1126.
Gianinazzi S, Gollotte A, Binet M, van Tuinen D, Redecker D and Wipf D, 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza, 20, 519–530.
Gonzalez-Teuber M, Kaltenpoth M and Boland W, 2014. Mutualistic ants as an indirect defence against leaf pathogens. New Phytologist, 202, 640–650.
Griffiths BS, Bengough AG, Neilson R and Trudgill DL, 2002. The extent to which nematode communities are affected by soil factors - a pot experiment. Nematology, 4, 943–952.
Jones CG, Lawton JH, Shachak M, Samson FB and Knopf FL, 1996 (reprinted). Organisms as ecosystem engineers. Oikos, 69, 373–386.
Jungbluth JH, Knorre DV. 2009. Rote Liste der Binnenmollusken [Schnecken (Gastropoda) und Muscheln (Bivalvia)] in Deutschland - 6. revidierte und erweiterte Fassung 2008. Mitteilungen der deutschen malakozoologischen Gesellschaft 81: 1–28.
Kammenga J, Arts M and Doroszuk A, 2000. Multigeneration effects at the population level: fitness maximization and optimal allocation in a nematode. In: Demography in ecotoxicology. Ed R KJaL, John Wiley, 164–177.
Kernaghan G, 2005. Mycorrhizal diversity: Cause and effect? Pedobiologia, 49, 511–520.
Khan Z and Kim YH, 2007. A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Applied Soil Ecology, 35, 370–379.
Kibblewhite MG, Ritz K and Swift MJ, 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B-Biological Sciences, 363, 685–701.
Koordinationsstelle Biodiversitäts-Monitoring Schweiz. 2009. Zustand der Biodiversität in der Schweiz. Ergebnisse des Biodiversitäts-Monitorings Schweiz (BDM) im Überblick. Stand: Mai 2009. Umwelt-Zustand Nr. 0911. Bern: Bundesamt für Umwelt. 104 pp.
Kuzyakov Y and Blagodatskaya E, 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology & Biochemistry, 83, 184–199.
Lavelle P and Spain AV, 2005. Soil Ecology. Eds Lavelle P and Spain AV. Springer, Dordrecht, The Netherlands, 654 pp.
Lavelle P, 1996. Diversity of soil fauna and Ecosystem Function. Biology International 33.
Luc M, Sikora RA and Bridge J, 2005. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Ed n. CABI Publishing, Wallingford, UK, 871 pp.
Manachini B, Corsini A and Bocchi S, 2009. Soil quality indicators as affected by a long term barley-maize and maize cropping systems. Italian Journal of Agronomy, 4, 15–22.
McElroy TC and Diehl WJ, 2001. Heterosis in two closely related species of earthworm (*Eisenia fetida* and *E. andrei*). Heredity, 87, 598–608.
McSorley R, Wang KH and Church G, 2008. Suppression of root-knot nematodes in natural and agricultural soils. Applied Soil Ecology, 39, 291–298.
Nagy P. 2009. Case studies using nematode assemblage analysis in terrestrial habitats. Eds Wilson M and Kakouli-Duarte T. Nematodes As Environmental. Indicators. CABI, Wallingford, UK, 15 pp.

Okada H and Harada H, 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Applied Soil Ecology, 35, 582–598.
OECD (Organization for Economic Co-operation and Development). Earthworm, Acute Toxicity Tests (No. 207). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
Shashikumar S and Rajini PS, 2010. Cypermethrin elicited responses in heat shock protein and feeding in *Caenorhabditis elegans*. Ecotoxicology and Environmental Safety, 73, 1057–1062.
Swarowsky K, Brühl C, Stahlschmidt P, 2013. The importance of field margins and meadows for land snails in the agricultural landscape. Mainzer Naturwissenschaftliche Archive, 50, 291–300.
Thimm T, Hoffmann A, Borkott H, Munch JC and Tebbe CC, 1998. The gut of the soil microarthropod *Folsomia candida* (Collembola) is a frequently changeable but selective habitat and a vector for microorganisms. Applied and Environmental Microbiology, 64, 2660–2669.
Van der Heijden MGA, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A and Sanders IR, 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytologist, 172, 739–752.
Van der Heijden MGA, Bardgett RD and van Straalen NM; 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11, 296–310.
Wall DH, Bardgett RD, Behan-Pelletier V, Herrick JE, Jones H, Ritz K, Six J, Strong DR and Van der Putten VH, 2012. Soil Ecology and Ecosystem Services. Eds Wall DH, Bardgett RD, Behan-Pelletier V, Herrick JE, Jones H, Ritz K, Six J, Strong DR and Van der Putten VH. Oxford University Press, UK, 405 pp.
Wang B and Qiu YL, 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16, 299–363.
Weinert N, Piceno Y, Ding G-C, Meincke R, Heuer H, Berg G, Schloter M, Andersen G and Smalla K, 2011. PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. Fems Microbiology Ecology, 75, 497–506.
Willecke S. 1983. Zur Verteilung von Gehäuseschnecken in Weinbergen und angrenzenden Flächen. Verhandlungen der Gesellschaft für Ökologie 10: 103–114.
Yeates GW and Williams PA, 2001. Influence of three invasive weeds and site factors on soil microfauna in New Zealand. Pedobiologia, 45, 367–383.

Appendix B – Time of development of some worms belonging to the family Lumbricidae*

Species	No of cocoons per worm	Incubation time of cocoons (weeks)	Period of growth per worm (weeks)	Total time for development (weeks)	Growth to maturity (development of clitellum) (days)	Cocoon viability (%)	Reference
Eisenia fetida	11 (per year)	11	55	66			Edwards and Bohlen, 1996
	5.5 (per week)	–	–	–	–		Lavelle and Spain, 2005
	3.7 (per month)	7.7	–	–	–	81.9	Tripathi and Bhardwaj, 2004
	3.5 (over 10 days)	3.3 at 25°C	–	–	–		Venter and Reinecke, 1988
Dendrobaena subrubicunda	42	8.5	30	38.5			Edwards and Bohlen, 1996
Lumbricusrubellus	106	16	37	53			Edwards and Bohlen, 1996
Lumbricus castaneus	65	14	24	38			Edwards and Bohlen, 1996
Aporrectodea rosea	8	17.5	55	72.5			Edwards and Bohlen, 1996
Aporrectodea caliginosa	27	19	55	74			Edwards and Bohlen, 1996
	31.2 at 15°C	33.4 at 5°C; 17 at 10°C; 8.8–12 at 15°C; 5.1 at 20°C	–	–	–	90 at 20°C	Lowe and Butt, 2005
Allolobophora chlorotica	27	12.5	36	48.5			Edwards and Bohlen, 1996
	9.9 at 10°C, 27.3 at 20°C	~ 60 at 5°C; 13–25 at 10°C; 7.3–8.4 at 15°C; 10 at 20°C, 4.9–5.7 at 25°C			84 at 15°C; 56 at 20°C	54 at 10°C; 62 at 15°C; 65–90 at 20°C	Lowe and Butt, 2005
Aporrectodea longa	8	10	50	60			Edwards and Bohlen, 1996
	17 at 20°C	15 at 9.6°C; 7.7–8.7 at 15°C; 5.3–8 at 20°C; 6 at 26°C			168 at 15°C; 120 at 20°C	70 at 15°C; 47 at 20°C	Lowe and Butt, 2005
Octodrilus complanatus	52	9.4 (in the range 7–10)		–	150	55	Monroy et al., 2007

Species	No of cocoons per worm	Incubation time of cocoons (weeks)	Period of growth per worm (weeks)	Total time for development (weeks)	Growth to maturity (development of clitellum) (days)	Cocoon viability (%)	Reference
Aporrectodea trapezoides	105	–	–	–	–	87	Fernandez et al., 2010
Lumbricus terrestris	25.3 at 15°C; 36.9 at 15°C (field) 10.1 at 20°C (lab)	39 at 5°C; 26 at 10°C; 10 at 20°C; 11.6 at 25°C			213 at 7.5°C; 112 at 15°C; 90 at 20°C	70 betw. 5–20°C; 83 at 15 °C; 41 at 25°C	Lowe and Butt, 2005
Octolasion cyaneum	32.3 at 20°C	16.3 at 15°C; 12.3 at 20°C				79 at 15°C; 77 at 20°C	Butt, 1993

*: Please, consider that the data reported are not intended to be exhaustive but only indicative and that the reproduction efficiency is highly dependent on factors like quality of the food, moisture content and temperature of the growth substrate, etc.

References

Butt KR, 1993. Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. Biology and Fertility of Soils, 16, 135–138.

Edwards C and Bohlen P, 1996. Biology and ecology of earthworm. 3rd edn, Chapman and Hall, London, 426 pp.

Fernandez R, Novo M, Gutíerrez M, Almodóvar A, Díaz Cosín DJ, 2010. Life cycle and reproductive traits of the earthworm *Aporrectodea trapezoides* (Duges, 1828) in laboratory cultures. Pedobiologia, 53, 295–299.

Lavelle P and Spain AV, 2005. Soil Ecology. Springer.

Lowe C and Butt K, 2005. Culture techniques for soil dwelling earthworms: A review. Pedobiologia, 49, 401–413.

Monroy F, Aira M, Gago J and Domínguez J, 2007. Life cycle of the earthworm Octodrilus complanatus (Oligochaeta, Lumbricidae). Comptes Rendus Biologies, 330, 389–391.

Tripathi G and Bhardwaj P, 2004. Comparative studies on biomass production, life cycles and composting efficiency of *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). Bioresource Technology, 92, 275–283.

Venter J and Reinecke A, 1988. The life-cycle of the compost worm *Eisenia fetida* (Oligochaeta). South African Journal of Zoology, 23, 161–165.

Appendix C – Overview of recovery potential for soil microorganisms

Table C.1: List of studies reporting significant increase in the measured endpoint compared to the control followed by no differences (from Puglisi, 2012)

Substance	Substance dose		Time to recover (days)	Total experiment time (days)	Method
Iprodione	0.83 (AR)[a]	mg/kg	60	90	FDA[b]
Iprodione	8.3 (10X AR)[c]	mg/kg	60	90	FDA
Propargyl Bromide	79 (AR)	mg/kg	28	90	Dehydrogenase
Mefenoxam	4 (AR)	mg/kg	60	60	MBC[d]
Copper Oxide	128 (AR)	mg/kg	60	60	MBC
Fenvalerate	1 (NR)[e]	mg/kg	35	35	Catalase
Fenvalerate	10 (NR)	mg/kg	35	35	Catalase
Fenvalerate	40 (NR)	mg/kg	35	35	Catalase
Fenvalerate	80 (NR)	mg/kg	35	35	Catalase
Chlorpyrifos	1 (NR)	mg/kg	35	35	Catalase
Chlorpyrifos	10 (NR)	mg/kg	35	35	Catalase
Chlorpyrifos	40 (NR)	mg/kg	35	35	Catalase
Chlorpyrifos	80 (NR)	mg/kg	35	35	Catalase
Bromacil	80 (NR)	kg/ha	28	28	Phosphatase
Mefenoxam	72 (AR)	μg a.i./100 g	120	120	Dehydrogenase
Mefenoxam	72 (AR)	μg a.i./100 g	120	120	Dehydrogenase
Mefenoxam	72 (AR)	μg a.i./100 g	120	120	Alkaline phosphatase
Mefenoxam	72 (AR)	μg a.i./100 g	90	120	Acid phosphatase
Diazinon	800 (NR)	g a.i./ha	60	150	Arginine deaminase
Endosulfan	1 (NR)	mg/kg	98	98	FDA
Endosulfan	10 (NR)	Mg/kg	98	98	FDA
Endosulfan	1 (NR)	mg/kg	98	98	Arylsulfatase
Fenpropimorph	1.3 (AR)	mg/kg	30	56	CFU[f] of total bacteria
Diazinon	800 (NR)	g a.i./ha	60	150	Dehydrogenase
Hexachlorocyclohexane	7.5 (AR)	kg a.i./ha	45	60	CFU of N-fixing bacteria
Phorate	1.5 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Carbofuran	1 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Fenvalerate	0.35 (AR)	kg a.i./ha	60	60	CFU of N-fixing bacteria
Captan	0.125	g a.i./kg	45	65	MBC

Substance	Substance dose		Time to recover (days)	Total experiment time (days)	Method
Triasulfuron	5 (> AR)	mg a.i./kg	50	56	Soil respiration
Triasulfuron	5 (> AR)	mg a.i./kg	50	56	Dehydrogenase
Primisulfuron methyl	5 (> AR)	mg a.i./kg	50	56	Soil respiration
Primisulfuron methyl	5 (> AR)	mg a.i./kg	50	56	Dehydrogenase
Rimsulfuron	5 (> AR)	mg a.i./kg	18	56	Soil respiration
Rimsulfuron	5 (> AR)	mg a.i./kg	18	56	Dehydrogenase

(a): AR: Application rate according to Good Agricultural Practice.
(b): FDA: Fluorescent diacetate hydrolytic activity.
(c): 10X AR: 10 times the application rate according to Good Agricultural Practice.
(d): Microbial biomass carbon.
(e): NR: not reported. It is not clear from the original study whether the used concentration were chosen according to good agricultural studies.
(f): Colony forming units.

Table C.2: List of studies reporting significant decrease in the measured endpoint compared to the control followed by no differences (from Puglisi, 2012)

Substance	Substance dose		Time to recover (days)	Total experiment time (days)	Method
Quinalphos	4 (AR)	L/ha	75	105	Dehydrogenase
Quinalphos	4 (AR)	L/ha	30	105	Phosphatase
Propargyl Bromide	79 (AR)	mg/kg	28	90	Acid phosphatase
Chloropicrin	176 (AR)	mg/kg	14	90	Beta-glucosidase
Quinalphos	2,5 (NR)	mg/50 cm^2	90	90	Dehydrogenase
Quinalphos	2,5 (NR)	mg/50 cm^2	7	90	Alkaline phosphatase
Bensulfuron Methyl	51 (AR)	g/ha	1	8	Potential nitrification
Bensulfuron Methyl	510 (10X AR)	g/ha	8	8	Potential nitrification
Benomyl	51 (AR)	mg a.i./kg	14	56	Soil respiration
Captan	125 (AR)	mg a.i./kg	28	56	Soil respiration
Chlorothalonil	37 (AR)	mg a.i./kg	28	56	Soil respiration
Bensulfuron Methyl	0,1 (10X AR)	μg/g	7	45	Potential nitrification
Bensulfuron Methyl	1 (100X AR)	μg/g	7	45	Potential nitrification
Nanopropamide	20 (10X AR)	mg a.i./kg	14	56	Soil respiration
2-Phenylethyl Isothiocyanate	10 (NA)[(a)]	mg/kg	30	60	Microbial respiration
Metam Sodium	300 (AR)	mg/kg	30	60	Microbial respiration
Bensulfuron Methyl	51 (AR)	g/ha	8	8	CFU of total bacteria
Bensulfuron Methyl	510 (10X AR)	G/ha	8	8	CFU of total bacteria
Pentachlorophenol	9,5 (AR)	Kg/ha	8	8	CFU of total bacteria
Pentachlorophenol	95 (10X AR)	Kg/ha	8	8	CFU of total bacteria
Benomyl	51 (AR)	mg a.i./kg	56	56	MBN
Captan	125 (AR)	mg a.i./kg	56	56	MBN
Chlorothalonil	37 (AR)	mg a.i./kg	56	56	MBN
Chlorpyrifos	2 (AR)	mg/kg	3	28	CFU of total bacteria
Chlorpyrifos	2 (AR)	mg/kg	14	28	CFU of total fungi
Bensulfuron Methyl	0,01 (AR)	μg/g	5	45	Potential nitrification
Carbendazim	10 (NR)	mg/kg	3	3	Potential nitrification
Chloranil	10 (NR)	mg/kg	3	3	Potential nitrification
Chloroneb	10 (NR)	mg/kg	3	3	Potential nitrification
Chlorothalonil	10 (NR)	mg/kg	3	3	Potential nitrification

Substance	Substance dose		Time to recover (days)	Total experiment time (days)	Method
Dichlone	10 (NR)	mg/kg	3	3	Potential nitrification
Dodine	10 (NR)	mg/kg	3	3	Potential nitrification
Fenaminosulf	10 (NR)	mg/kg	3	3	Potential nitrification
Folpet	10 (NR)	mg/kg	3	3	Potential nitrification
Maneb	10 (NR)	mg/kg	3	3	Potential nitrification
Quintozene	10 (NR)	mg/kg	3	3	Potential nitrification
Thiram	10 (NR)	mg/kg	3	3	Potential nitrification
Zineb	10 (NR)	mg/kg	3	3	Potential nitrification
Chloranil	10 (NR)	mg/kg	1	2	Denitrification
Dichlone	10 (NR)	mg/kg	1	2	Denitrification
Fenaminosulf	10 (NR)	mg/kg	1	2	Denitrification
Thiram	10 (NR)	mg/kg	1	2	Denitrification
Zineb	10 (NR)	mg/kg	1	2	Denitrification
Chlorothalonil	5.4 (100X AR)	g a.i./kg	65	65	MBC
Carbendazim	45 (100X AR)	g a.i./kg	65	65	MBC
Forchlorfenuron	0.5 (AR)	mg/kg	15	30	Acid phosphatase
Butachlor	100 (NR)	mg/kg	21	21	CFU of total bacteria

(a): NA: not applicable. The substance was applied at different concentrations in order to mimick the field application of metam-sodium.

Table C.3: Overview of recovery potential for soil microorganisms. List of studies reporting transient effects I the measured endpoint compared to the control followed by no differences (from Puglisi, 2012)

Substance	Substance dose		Time to recover (days)	Total experiment time (days)	Method
Atrazine	1	mg/kg	30	60	PCR-DGGE[(a)] of bacterial 16S
Atrazine	2	mg/kg	30	60	PCR-DGGE of bacterial 16S
Atrazine	3	mg/kg	30	60	PCR-DGGE of bacterial 16S
Iprodione	5	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	5	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	50	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Iprodione	50	mg a.s./g	23	23	PCR-DGGE of bacterial 16S
Carbendazim	0.94	kg a.s./ha	360	360	TGGE of bacterial 16S
Carbendazim	1.88	kg a.s./ha	360	360	TGGE of bacterial 16S
Carbendazim	4.70	kg a.s./ha	360	360	TGGE of bacterial 16S
Acetochlor	50	mg/kg	56	56	PCR-DGGE of fungal communities
Acetochlor	150	mg/kg	56	56	PCR-DGGE of fungal communities
Acetochlor	250	mg/kg	56	56	PCR-DGGE of fungal communities
Butachlor	2	mg/kg	21	21	Biolog

(a): PCR-DGGE: Polymerase chain reaction-denaturing gradient gel electrophoresis.

Reference

Puglisi E, 2012. Response of microbial organisms (aquatic and terrestrial) to pesticides. EFSA Supporting Publications 2012:EN-359, 175 pp.

Appendix D – Summary of Dutch proposal for risk assessment of persistent substances

Persistence of plant protection products in soil is one of the aspects included in the evaluation of active substances and authorisation of PPPS. Under the former regulation at the EU level there was a general agreement on trigger values that indicate the need for further research, but there were different views on the assessment and the interpretation of this additional information at the national level. As a result, member states adopted different evaluation procedures. For example, the Netherlands included a cut-off value of 180 days for the dissipation half-life (DT_{50}) in soil. Since it appeared that this was not in line with the principles of the EU legislation, the Netherlands drafted a proposal for the risk assessment of persistence of PPPs in soil (Van der Linden et al., 2008). Most other countries in the EU did not use a cut-off value.

What are the protection goals?

The Dutch workgroup proposes to consider up to three principles to set protection goals for soil, each having its own timeframe:

Principle to set protection goal	Time scale
Functional Redundancy Principle (FRP)	In year of cropping
Community Recovery Principle (CRP)	2 year post-last application
Ecological Threshold Principle (ETP)	7 years post-last application

The goals are:

1) Protection of life-support functions of the in-crop soil to allow the growth of the crop and protection of key(stone) species (earthworms) of agricultural soils (FRP). Starting point is that this aspect will be included in the new guidance document anyway.
2) Protection of life-support functions of the soil to allow crop rotation and sustainable agriculture, with overall protection of the structure and functioning of soil communities characteristic for agro–ecosystems (CRP).
3) Protection of life-support functions of the soil to allow changes in land use, with overall protection of the structure and functioning of soil communities characteristic for nature reserves (ETP).
4) The approach has been developed for the in-crop area.

What are the trigger values?

The half-life for dissipation (DT_{50}) of a chemical from soil acts as a trigger value for evaluation according to one or more of the protection goals. Substances having a DT_{50} above 90 days at 10 °C are evaluated according to the CRP and substances having a DT_{50} above 180 days at 10 °C are additionally evaluated according to the ETP. The values trigger the assessment, but in general additional tests as well.

What is the principle of the risk assessment?

Predicted environmental concentrations (PEC_s) are compared to ecotoxicological relevant concentrations, for instance EC_{50} or NOEC values of indicator species. The assessment evaluates whether, in the realistic worst-case exposure, i.e. the 90th percentile, critical values of the exposure/toxicity ratio are exceeded. Substances which exceed the critical value cannot be authorised. The critical values are derived based on EU Technical Guidance Documents; sometimes with a pronounced preference for one of the given options. The assessment can be based both on the total content of the substance as well as on the pore water concentration.

What are the main elements of the assessment?

Both at the exposure side and at the ecotox side, a tiered approach is suggested: ranging from simple and conservative, using higher assessment factors, to more complex and realistic, with lower assessment factors. The first tier of the exposure assessment uses a scenario that is generically vulnerable to persistence. This scenario is run several times, with different input sets in order to ensure conservative results for both total content and on pore water concentration. The second tier of the exposure assessment uses a spatially distributed model so that the realistic worst case condition is

determined during the calculations. At the ecotox side, the CRP and ETP protection goals have separate ecotox assessment schemes, existing of three tiers each.

For the CRP 2 years after the last application *potential* recovery of sensitive soil populations of agro-ecosystems is assured (TER approach based on chronic lab toxicity tests with a basic set of in-soil organisms; SSD approach based on chronic tests and the median HC_5; field experiment approach).

For the ETP 7 years after last application exposure to the PPPs and its metabolites do not affect sensitive populations of in-soil organisms (TER approach based on chronic lab toxicity tests with a larger number of species or the application of a larger AF; SSD approach based on chronic lab toxicity tests and the lower limit of the HC_5 or the use of the median HC_5 value and the application of an AF; model ecosystem approach and extra AF).

Reference

Van der Linden A, Boesten J, Brock T, Van Eekelen G, Ter Horst M, De Jong F, Montforts M and Pol J, 2008. Revised proposal for the risk assessment of persistence of plant protection products in soil. RIVM report 601712003.

Appendix E – Background considerations to the section 'Temporal and spatial exposure profiles for in-soil organisms'

In Section 7.10.3 'Temporal and spatial exposure profiles for in-soil organisms', it is stated that it is crucial to predict accurately the type of environmental concentration which organisms are exposed to and that elicits the observed effects (ecotoxicologically relevant concentration, ERC) in order to properly assess the risks of plant protection products (PPP) intended uses. The potential effects of PPP on soil organisms depend – besides the concentration of the chemical in the soil profile – on the spatial and temporal distribution of the animals, i.e. their exposure as well as their specific sensitivity to the chemical.

To establish a relationship between exposure and effects, Toschki et al. (2015) conducted different outdoor studies in Terrestrial Model Ecosystems (TMEs) to monitor (A) the fate and behaviour of pesticides with different properties in soil over time and (B) the effects of PPP on soil organisms at the same time. Additionally, they conducted an indoor TME study to measure the fate of radiolabelled pesticides and the formation of non-extractable residues in soil over time. For the outdoor studies, pesticides with different physicochemical properties and mode of actions were applied; lindane (log K_{ow} > 3), imidacloprid (log K_{ow} < 1) and carbendazim (selected for known earthworm toxicity). For the outdoor studies, 113 TMEs (Ø 467 mm, height 400 mm) were set up, for the isotope-laboratory study, 10 TMEs (Ø 100 mm, height 400 mm, imidacloprid and lindane only).

PPP were applied at two rates in replicate TMEs. Every PPP had a 'high' and 'lower' application rate. In this respect, the applied pesticide amounts were chosen in order to certainly elicit effects on soil organisms, so that the distribution of effects could be studied in the soil profile. The applied rates were for lindane 7.5 and 20 kg a.s/ha; for imidacloprid 0.75 and 2 kg a.s./ha and for carbendazim 7.5 and 15 kg/ha. The applied pesticide amounts were not chosen in relation to the approval of the respective active substances, but to possibly elicit effects on every soil organisms groups assessed (see Figure E.1). For details on the study design and sampling, please refer to Toschki et al. (2015). Some general features are given below.

For the microarthropods and enchytraeids, 5 sampling dates with 5 replicate TMEs for each concentration and 10 controls were set. For the earthworms, three sampling dates were set with 5 replicates for the lower rate of each pesticide and the control. At the end of the study, after all microarthropod, enchytraeid and analytical samples were taken, the soil of all TMEs, i.e. two pesticides with two concentrations each, were sampled for earthworms. Thus, for the last sampling date also data for earthworms at the higher pesticide rates were available. To sample earthworms, it was necessary to destructively sample the entire TME soil core at a time.

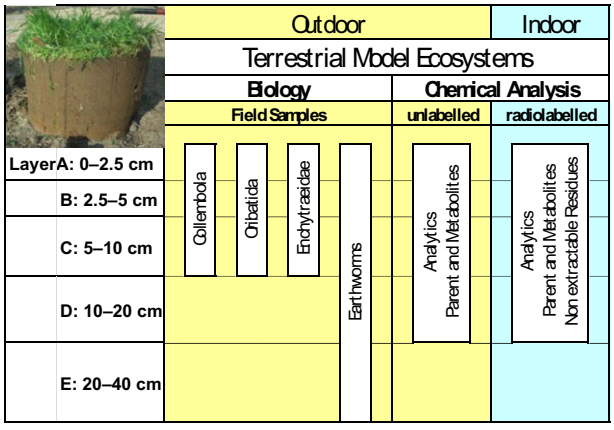

Figure E.1: Link of analytical and biological data out of the field and indoor laboratory by sampling in Terrestrial Model Ecosystem (TME). From Toschki et al. (2014, 2015)

We analysed the results of the TME study by Toschki et al. (2015) to detect the exposure assessment which would best link the observed effects of the tested active substances on soil organisms.

To do so, we predicted effects of the studied PPPs on soil organisms using available effect data from laboratory experiments (dose–response curves) and related those to average concentrations measured in different layers of the TMEs. The question was which effect class would be expected in

the different layers of the TMEs by the measured concentrations. Then the observed effects in the different soil depths of the TMEs according to Toschki et al. (2015) are compared to the measured exposure concentrations, in order to possibly relate exposure and effect in the different TME soil depths.

A second type of analysis regards the possible decision on which soil depth to choose to average soil volume for the calculation of predicted environmental concentrations (PEC) in soils. EFSA (2009a) and EFSA PPR Panel (2010) suggest to calculated PEC differently depending on the soil horizon depth inhabited by different soil organisms' groups. As general rule, soil microarthropods are thought to be exposed to an average concentration in the first 2.5 cm of the soil profile, while earthworms are thought to be exposed to an average concentration over 20 cm. These hypotheses are investigated here for some example taxa investigated in the TME studies of Toschki et al. (2015). All calculation and presented examples were performed with measured effect and exposure data of the TMEs treated with the lower of the two concentrations of lindane, imidacloprid and carbendazim, respectively (Toschki et al., 2015).

Lindane

As predicted by the mode of action of the active substance, the tested concentrations of lindane did not elicit acute effects on earthworms. As expected, Collembola were highly impacted by the applied amount of lindane. By way of example, this soil organism group was chosen for further analysis. But, since the observed effect in the lowest application rate exceeded initially the 95% mark in some horizons, the exact effect expression and its spatial distribution cannot be described properly for this substance. Nevertheless, it can be seen from the table below that the concentration of lindane in the upper soil layer analysed (0–2.5 cm) best explains the observed effects on Collembola abundance in all layers of the entire soil column.

The observed high effects on collembola individual numbers in lower TMEs layers cannot be explained by the concentration in the respective layer were they have been sampled –but only by active substance concentrations in the upper 2.5 cm horizon. In the indoor experiment with radiolabelled lindane, more than 85% of the active was detected in the 0–1 cm horizon up to sampling day 42 (2nd sampling after treatment). Together with the sampling horizon 1–2.5 cm, 95% of the

Table E.1: Lindane – observed versus predicted effects on Collembola in TME soil sampling layers. Measured concentrations and respective effect data from Toschki et al. (2015)

Layer (cm)	Measured concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the different TME layers at 2nd sampling after treatment	Predicted effects according to lab toxicity*
0–2.5	28.40	98%	> **13*LC_{50} ~ 100%**
2.5–5	0.31	80%	< **50%**
5–10	0.02	90%	< **LOEC**
10–20	0	Not assessed	

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *F. candida* exposed to Lindane in the laboratory (Lock et al., 2002), LOEC (28 days) = 0.056–0.1 mg a.s./kg dw soil, LC_{50} (4 weeks) = 0.363–2.21 mg a.s./kg dw soil.

active substance remained in the first layer 0–2.5 of the TMEs.

To evaluate, up to which depth the soil concentration might be averaged starting at the surface for the calculation of predicted environmental concentrations, PEC in soil depths of 0–1, 0–5, 0–10 and 0–20 cm (as suggested by EFSA, 2009a,b,c, 2010) were calculated based on the concentrations

Table E.2: Lindane – observed versus predicted effects on Collembola in TME soil sampling layers. Effect data from Toschki et al. (2015), predicted concentrations are calculated averaged over different depths as suggested by EFSA (2010)

Layer (cm)	Calculated average concentrations in TMEs at day 1 after treatment (mg/kg soil)		Observed effects in the different TME layers at 2nd sampling after treatment			Predicted effects according to lab toxicity*
			0–2.5	2.5–5	5–10	
0–1	71.0	**	98%	80%	90%	**> 32*LC_{50} ~ 100%**
0–2.5	28.4					**> 13*LC_{50} ~ 100%**
0–5	14.7	***				**> 7*LC_{50} ~ 50–100%**
0–10	7.1	***				**> 3.2*LC_{50} ~ 50–100%**
0–20	3.6	***				

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *F. candida* exposed to Lindane in the laboratory (Lock et al., 2002), LOEC (28 days) = 0.056–0.1 mg a.s./kg dw soil, LC_{50} (4 weeks) = 0.363–2.21 mg a.s./kg dw soil.
**: Calculated assuming that the entire amount of active substance stays in the first cm.
***: Calculated by adding up measured concentration levels and averaging over total soil depth.

measured in the TME (see table above) and subsequently averaged. Predicted effects in these layers were compared to observed effects in the TMEs reported by Toschki et al. (2015).

As reported above, the clear overdosage of lindane in the TME resulted in effects on Collembola densities partly higher than 90%. Therefore, the measured concentrations in the TMEs were too high to accurately assess which averaged concentration in the soil (over a profile starting at the soil surface) best predicted the effects. The table shows that any of the averaged concentrations may have already predicted 100% effects.

Imidacloprid

As predicted by the mode of action of the active substance, the tested concentrations of imidacloprid did not elicit acute effects on earthworms. As expected, Collembola were highly impacted by the applied amount of imidacloprid and were chosen for further analysis.

For Collembola, it can be seen from the table below that the concentrations measured in the upper layer of the TMEs (0–2.5 cm) explains best the observed effects at the second sampling after treatment. Observed effects on Collembola in lower TME soil layers can only be explained by concentrations in the uppermost soil layer –and not by the measured concentrations in the layer were they were detected at time of sampling.

It should be noted that even the prediction based on the concentration in the 0–2.5 cm layer

Table E.3: Imidacloprid – observed versus predicted effects on Collembola in TME soil sampling layers. Measured concentrations and respective effect data from Toschki et al. (2015)

Layer (cm)	Measured concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the different TME layers at 2nd sampling after treatment	Predicted effects according to lab toxicity*
0–2.5	2.08	50%	**45%**
2.5–5	0.27	70%	**< 1%**
5–10	0	70%	**0%**
10–20	0	Not assessed	

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *F. candida* exposed to Imidacloprid SL 200 in artificial soil in the laboratory (please refer to DAR Imidacloprid, 2005). A log-logistic model (ED_{50} as parameter, LC_{50} 3.4 mg/kg) with lower limit at 0 (3 parameters) fitted to the data using [R] version 3.1.1, package drc version 2.5–12 (model LL.3, b = –2.7109, d = 0.9423, e = 2.2271).

underestimated the observed effects. This indicates that the ecotoxicologically relevant concentration level may be a higher concentration (e.g. as it is found in 0–1 cm soil depth in the indoor studies).

To evaluate, up to which depth the soil concentration might be averaged starting at the surface for the calculation of predicted environmental concentrations, PEC in soil depths of 0–1, 0–5, 0–10 and 0–20 cm were calculated based on the concentrations measured in the TME (see table above) and subsequently averaged. Predicted effects in these layers were compared to observed effects in the TMEs reported by Toschki et al. (2015). Given the underestimation of effects based on the concentration in the

Table E.4: Imidacloprid – observed versus predicted effects on Collembola in TME soil sampling layers. Effect data from Toschki et al. (2015), predicted concentrations are calculated averaged over different depths as suggested by EFSA (2010)

Layer (cm)	Calculated average concentrations in TMEs at day 1 after treatment (mg/kg soil)		Observed effects in the different TME layers at 2nd sampling after treatment			Predicted effects according to lab toxicity*
			0–2.5	2.5–5	5–10	
0–1	5.20	**	50%	70%	70%	**91%**
0–2.5	2.08					**45%**
0–5	1.18	***				**15%**
0–10	0.60	***				**3%**
0–20	0.30	***				**0.4%**

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *F. candida* exposed to Imidacloprid SL 200 in artificial soil in the laboratory (please refer to DAR Imidacloprid, 2005). A log-logistic model (ED_{50} as parameter, LC_{50} 3.4 mg/kg) with lower limit at 0 (3 parameters) fitted to the data using [R] version 3.1.1, package drc version 2.5-12 (model LL.3, b = −2.7109, d = 0.9423, e = 2.2271).
**: Not measured but calculated assuming that the entire amount of active substance stays in the first cm.
***: Calculated by adding up measured concentration levels and averaging over total soil depth.

0–2.5 cm layer, the estimated concentration in the 0–1 cm layer would more appropriately predicted effects on Collembola due to imidacloprid applied rates in the TMEs (see table below).

Carbendazim

The TME study by Toschki et al. (2015) with the active substance carbendazim was set up to explicitly test the application of this active on earthworms and the distribution of the effects in the different layers of the TME profile. This active poses a contrast in its expected effect spectrum compared to the other a.s. already investigated in the TMEs. Given the expertise gained in the TMEs with lindane and imidacloprid – which run in parallel but before this study – the sampling technique was improved and it was possible also in the outdoor mesocosms to sample as first layer 0–1 cm instead of 0–2.5 cm (please see table below).

Considering total abundance of earthworms in the different soil layers of the treated TMEs compared to controls, the concentration of carbendazim in 0–1 and 0–2.5 cm depth allowed the best

Table E.5: Carbendazim – observed versus predicted effects on Earthworms in TME soil sampling layers. Measured concentrations and respective effect data from Toschki et al. (2015)

Layer (cm)	Measured concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the different TME layers at 2nd sampling after treatment	Predicted effects according to lab toxicity*
0–1	8.87		**99.9%**
0–2.5	4.50	82%	**95.9%**
2.5–5	0.18	61%	**< 1%**
5–10	0	45%	**0%**
10–20	0	50%	**0%**
20–40	Not measured ~ 0	65%	**---**

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *Eisenia fetida* (Rico et al. 2016). LC_{50} = 2 mg a.s./kg soil; LC_{10} = 1.1 mg a.s./kg soil, probit model fitted to data, slope = 2.14364712107952, intercept = −1.48586295809171.

estimation of the observed effects at the second sampling after treatment (here day 144). The observed high to medium effects on earthworms in deeper soil layers of the TME could not be explained by the measured concentrations in the respective layers.

To evaluate, up to which depth the soil concentration might be averaged starting at the surface (as

Table E.6: Carbendazim – observed versus predicted effects on Collembola in TME soil sampling layers. Effect data from Toschki et al. (2015), predicted concentrations are calculated averaged over different depths as suggested by EFSA (2010)

Layer (cm)	Calculated average concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the different TME layers at 2nd sampling after treatment					Predicted effects according to lab toxicity*
		0–2.5	2.5–5	5–10	10–20	20–40	
0–1	8.87	82%	61%	48%	50%	65%	**99.9%**
0–2.5	4.50						**95.9%**
0–5	2.34						**63.2%**
0–10	1.17						**12.5**
0–20	0.60						**0.5**

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *Eisenia fetida* (Rico et al. 2016). LC_{50} = 2 mg a.s./kg soil; LC_{10} = 1.1 mg a.s./kg soil, probit model fitted to data, slope = 2.14364712107952, intercept = −0.48586295809171.

done in the current risk assessment), concentrations in depths of 0–1, 0–5, 0–10 and 0–20 cm were calculated based on the measurements by Toschki et al. (2015) presented above. Predicted effects in these layers were compared to observed effects in the TMEs.

Based on this evaluation, it can be seen that concentrations predicted in 0–5 cm depth allowed the best estimation of effects in deeper layers and the PEC in 0–2.5 cm depth allowed the best estimate of effects on total abundance activity in the same soil layer. Effects observed in deeper soil depths could not be explained by the concentrations averaged over 20 cm. As shown in the table above, calculating for earthworms an average distribution depth of the active substance in 20 cm depth would have resulted in a concentration (here 0.6 mg carbendazim/kg soil dw) suggesting that the expected effects would range less than 1% compared to control. However, the effects on earthworm abundance seen in the TME study by Toschki et al. (2015) were in the range of a medium lethal concentration (here between 50% and 65% effect).

The earthworm species pooled in the data analysed in the previous table belong to different groups: anecic, endogeic and epigeic species. Their different behaviour and their occurrence in different soil depths as well as differences in sensitivity make are not appropriately analysed based on total abundance. Therefore, we analysed separately the exposure-effect relationship for different species.

Table E.7: Carbendazim – observed versus predicted effects on Earthworms in TME soil sampling layers. Effect data for single species from Toschki et al. (2015), predicted concentrations are calculated averaged over different depths as suggested by EFSA (2010)

***Lumbricus castaneus* (epigeic species)**

Layer (cm)	Calculated average concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the single TME layers at 2nd sampling after treatment**					Predicted effects according to lab toxicity*
		0–2.5	2.5–5	5–10	10–20	20–40	
0–1	8.87	100%		100%			99.0%
0–2.5	4.50						95.9%
0–5	2.34						63.2%
0–10	1.17						12.5%
0–20	0.60						0.5%

Layer (cm)	Calculated average concentrations in TMEs at day 1 after treatment (mg/kg soil)	Observed effects in the single TME layers at 2nd sampling after treatment**					Predicted effects according to lab toxicity*
		0–2.5	2.5–5	5–10	10–20	20–40	
***Lumbricus castaneus* (epigeic species)**							
Aporrectodea caliginosa (endogeic species)							
0–1	8.87	89%	84%	14%	50%		99.0%
0–2.5	4.50						95.9%
0–5	2.34						63.2%
0–10	1.17						12.5%
0–20	0.60						0.5%
Octolasium cyaneum (endogeic species)							
0–1	8.87			100%	83%		99.0%
0–2.5	4.50						95.9%
0–5	2.34						63.2%
0–10	1.17						12.5%
0–20	0.60						0.5%
Lumbricus terrestris (anecic species)							
0–1	8.87				100%	100%	99.0%
0–2.5	4.50						95.9%
0–5	2.34						63.2%
0–10	1.17						12.5%
0–20	0.60						0.5%

Appropriate effect estimate.
Underestimation of effects.
*: Lab toxicity: calculations based on a laboratory study with *Eisenia fetida* (Rico et al. 2016). LC_{50} = 2 mg a.s./kg soil; LC_{10} = 1.1 mg a.s./kg soil, probit model fitted to data, slope = 2.14364712107952, intercept = −1.48586295809171.
**: Total number of individuals of the respective species captured in TMEs ≥ 4.

It can be seen from the table above that concentrations in the upper soil layer (0–2.5 cm and 0–1 cm) best explain the observed effects in the respective soil layers – but also the effects in the deeper soil layers. This is the case for the epigeic species *Lumbricus castaneus* that inhabits the upper soil layer, for the tested endogeic species that move in the whole soil profile and for the anecic species *Lumbricus terrestris* that prefers lower soil layers and come to the surface to feed.

Picoxystrobin

Picoxystrobin is a broad-spectrum fungicide belonging to the strobilurin group of chemicals. Strobilurins are part of the QoI group of fungicides.

Several field studies were included in the dossier submitted in the context of the active substances approval and summarised in the Renewal Assessment Reports (EFSA, 2016)[12] aiming at refining the risk to earthworms identified at tier 1.

Soil samples were analysed for residues of picoxystrobin and the results showed that the highest residues of picoxystrobin were detected in the topsoil profile (0–1 cm) indicating that the substance does not distribute through the soil profile.

With regard to the effects observed in field studies, it was noted that the most affected species were endogeic and anecic earthworms. Those group of earthworms, however, pass their time in the top 20 cm of the soil profile or the in permanent burrow systems that may extend several metres into the soil, respectively, while forging to the soil surface for food and water. However, effects observed in deeper soil depths could not be explained by the concentrations averaged over 20 cm as also demonstrated in the experiment by Toschki et al. (2015).

[12] http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4515/full
http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?1

Conclusions

Overall, the presented results show that in most cases the concentrations in the upper soil layer (0–2.5 cm or even 0–1 cm) explain the effects on in-soil organisms best. Only in the case of the TME study with lindane this rule was not met: here the chosen application rates were clearly too high, eliciting effects close to 100%. In such cases, it is not possible to precisely relate effects to predicted or measured concentrations, since no effect above 100% can be observed, while the concentrations measured suggest even stronger consequences.

The measured concentrations in the upper soil layers were also relevant for species that preferably live most of the time in deeper soil layers. Effects observed in these deeper layer were consistently related to concentrations only available in the upper soil layer. This is possibly explained by the high mobility of soil organisms species, i.e. movement of species at some times to the soil surface or upper layers for feeding, etc. In these cases, the highest concentration in the soil layer is relevant for assessing the risk. The averaged concentrations over an horizon of 10 or even 20 cm soil depth never delivers a predicted environmental concentrations able to explain any of the observed effects for different soil organisms – including earthworms – in any of the soil layers.

References

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2010. Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. EFSA Journal, 8, 77 pp.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA on the usefulness of total concentrations and pore water concentrations of pesticides in soil as metrics for the assessment of ecotoxicological effects. EFSA Journal, 922, 1–90.

EFSA (European Food Safety Authority), 2016. Peer review of the pesticide risk assessment of the active substance picoxystrobin. EFSA Journal, 14, 263 pp.

Rico A, Sabater C, Castillo MA, 2016. Lethal and sub-lethal effects of five pesticides used in rice farming on the earthworm *Eisenia fetida*. Ecotoxicology and Environmental Safety, 127, 222–229.

Toschki A, Hommen U, Klein K, Koenig W, Pieper S, Römbke R, Ross-Nickoll M, Schäffer A, Possberg C, Scholz-Starke B and M. Hammers-Wirtz, 2014. Evaluation of the risk for soil organisms under real conditions. Proceedings of the SETAC Europe 24th Annual meeting, Basel, Switzerland.

Toschki A, Hammers-Wirtz M, Poßberg C, Roß-Nickoll M, Schäffer A, Schmidt B, Scholz Starke B, Römbke J, Scheffzyk A, Klein M, Hommen U, 2015. Evaluation of the risk for soil organisms under real conditions - Development of a national position to consider the amending downstream legislations of the new EU Plant Protection Products Regulation for Pesticides. Report FKZ 3710 67 410 for the German Federal Environment Agency (UBA).

Appendix F – Summary of (extended) laboratory test systems with invertebrates identified as potentially relevant by the working group

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Non-arthropod invertebrates					
Earthworms *Eisenia fetida fetida/ andrei*	Earthworm reproductive toxicity test 56 days: 28 days exposure of adults; additional 28 days offspring and juveniles artificial soil (10% OM (Sphagnum peat), can according to OECD222 be lowered to 5%. Food (e.g. cow manure) is mixed with the soil source (ISO) or added to soil surface 1 day after application of test substance (OECD)	Reproduction NOEC, EC_x mg/kg active substance per dry weight soil (total content, nominal)	Amount of test substance applied is verified by a suitable calibration technique. Contact exposure via soil and pore water, oral exposure via soil and pore water, oral exposure via food Substance is mixed with the soil. Note I		ISO (2012) (11268-2)/OECD (2004) (222)
Mollusc *Helix aspersa*	28 days; semistatic (substrate or soil is renewed every 7 days)	Survival, growth	Food and contact		ISO (2006) (15952)
Earthworms *Eisenia fetida/ Eisenia andrei*	Earthworm acute toxicity test; 14 days Artificial soil 10% OM (Sphagnum peat)	Survival; EC_{50}; mg kg^{-1} active substance per dry weight soil (total content, nominal)	Note 1		ISO (2012) (11268-1)/OECD (1984) (207)
Nematodes *Caenorhabditis elegans*	Acute toxicity test 24–48 h	Survival; LC_{50}, EC_{50}			ASTM (2014) (E2172)
Nematodes *Caenorhabditis elegans*	Chronic toxicity test 96 h	Growth, reproduction; EC_{50}	Bulk soil, pore water and food (bacteria)		ISO (2010) (10872)
Enchytraeids *Enchytraeus albidus*	Enchytraid reproduction test 21 days (mortality adults) 42 days juveniles artificial soil (10% OM (Sphagnum peat) Test containers 0.02–0.25 L, 20 g dry weight soil. Feeding 50 mg of ground rolled oats before introducing the worms. Thereafter, 25 mg food is supplied weekly up to day 21	Survival, reproduction NOEC, EC_x mg/kg substance per dry weight soil (total content, nominal)	Note 1		OECD (2004) (220)/ISO (2014) (16387)
Enchytraeids *Cognettia sphagnetorum*	Enchytraid reproduction test 70 days Modified LUFA soil: 75% sphagnum peat soil: 25 vol% LUFA 2.2 soil (3,9% OM and 5,1% clay) Feeding by adding 1% algae or 0.2% baker's yeast	Survival, growth, reproduction	Note 1		Rundgren and Augustsson, chapter 6 in Løkke and Van Gestel (1998)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Enchytraeids *Enchytraeus crypticus*	Enchytraeid Reproduction Test[PLUS] Test follows standard guidelines (OECD, 2004 (220)/ISO, 2014 (16387)) with adaptations for (a) hatching, (b) growth and (c) full life cycle	(a) Hatching (b) Growth/ reproduction (c) Hatching, growth, survival, maturity status, reproduction, cocoon production, population growth rate (coccoon), population growth rate (juveniles)	Note 1		Bicho et al. (2015)
Arthropods					
Collembola *Folsomia candida/ Folsomia fimetaria*	Collembolan Reproduction Test in Soil 21 days (*F. fimetaria*), 28 days (*F candida*) artificial soil (5% Sphagnum peat) Test containers, 30 g soil (dry weight) Feeding at day 0 and day 14, 2–10 mg of granulated dry yeast is added to each test container	Survival Reproduction relative to control mg/kg as active substance per dry soil (nominal, total content	Note 1 Guideline gives the option of application of test substance to soil surface		ISO (2014) (11267)/OECD (2009) (232)
Collembola *Folsomia candida*	The methodology describes an acute toxicity test for Collembola in an aqueous medium in 100 mL sample vials. Effects expected from exposure in pore water can be assessed using this test method Concentrations were measured several times which allows for the generation of reliable and accurate dose–response curves	Survival	Test substance in aqueous medium to study exposure in pore water	Mortality was difficult to determine	Houx et al. (1996)
Collembola *Folsomia candida*	Collembola multigeneration test Collembola test is conducted as the standard test (ISO, 2014 (11267)/OECD, 2009 (232)), survival and reproduction tested. Only the F_0 generation is exposed to fresh residues. Effects on the following generations can be studied	Survival Reproduction, multi generation	Note 1 Standard guideline gives the option of application of test substance to soil surface		Campiche et al. (2007), Ernst et al. (2016)
Collembola *Sminthurus viridis, Folsomia candida, Isotomurus palustris, Isotoma viridis*	Bioassay: Collembola were contained in the laboratory for 24 h on presprayed soils aged for varying times in the field Assessment of the sensitivity of different species Experiments were conducted on field soil and lufa 2.2 soil	Survival	Exposure by spraying, deposition measured using water sensitive paper	The approach could be adapted to for multi-rate dose response testing	Wiles and Frampton (1996)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Mites *Hypoaspis* (*Geolaelaps*) *aculeifer*	*Hypoaspis aculeifer* Canestrini 14 days artificial soil (5% Sphagnum peat) As a food source the mite *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) is used	Survival reproduction Both endpoints as % reduction, relative to control, at a certain nominal dose rate (mg/kg)	Note 1		OECD (2008) (226)
Oribatid mite *Platynothrus peltifer*	14–70 days	Survival Reproduction			Van Gestel and Doornekamp, chapter 8 in Løkke and Van Gestel (1998)
Oribatid mite *Oppia nitens*	28 days	Reproduction			Princz et al. (2010)
Insect larvae *Oxythyrea funesta*	10 days acute toxicity test Artificial soil, 10% OM (Sphagnum peat), Cow dung is added as food for the larvae (3 g at start, 2–3 g after 3 and 7 days)	Survival; LC_{50}	Note 1		ISO (2005) (20963)
Centipede *Lithobius mutabilis*	Acute toxicity test 28 days for degradable compounds, 84 days for persistent chemicals Feeding two pupae of housefly a week. Larvae are fed with substrate including the test substance	Survival; LC_{50} Growth, respiration rate and locomotive activity (EC_{20}, EC_{50})	Note 1 Artificial soil (OECD), 10% OM (Sphagnum peat)		Laskowski, Pyza, Maryanski and Niklinska, chapter 11 in Løkke and Van Gestel (1998)
Millipede *Brachydesmus superus*	Chronic test 70 days Food supplied as decomposed leaf litter as polls and baker's yeast	Survival (EC_{50})reproduction	Note 1 LUFA 2.2		Tajovsky, chapter 12 Løkke and Van Gestel (1998)
Isopods *Porcellio scaber*	28 days	Survival, growth			Hornung, Farkas and Fischer in Løkke and Van Gestel (1998)
Isopods *Porcellionides pruinosis*	14	Survival, reproduction			Jänsch et al. (2005)
Carabid beetles *Pterostichus oblongopunctatus; Poecilus cupreus*	Different durations; Adult or larval	Survival; adult behaviour, respiration			Schrader et al. (1998), Bednarska et al. (2010)

Note 1: (a) test substance in deionised water mixed with artificial soil.
(b) if insoluble in water as (a) but test substance dissolved in a volatile organic solvent and mixed with a portion of the medium.
(c) if test substance not soluble, dispersible or emulsifiable, mixed with quartz sand, then mixed with artificial soil.

References

ASTM (American Society for Testing and Materials), 2014. Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode *Caenorhabditis elegans*, E2172. In: Annual Book of ASTM Standards, Environmental Assessment, Risk Management and Corrective Action; Biological Effects and Environmental Fate, 11.06.

Bednarska AJ, Gerhardt A and Laskowski R, 2010. Locomotor activity and respiration rate of the ground beetle, Pterostichus oblongopunctatus (Coleoptera: Carabidae), exposed to elevated nickel concentration at different temperatures: novel application of Multispecies Freshwater Biomonitor®. Ecotoxicology, 19, 864–871.

Bicho, R.C., Santos, F.C.F., Gonçalves, M.F.M., Soares, A.M.V.M., Amorim, M.J.B. (2015): Enchytraeid reproduction testPLUS: hatching, growth and full life cycle test – an optional multi-endpoint test with *Enchytraeus crypticus*. Ecotoxicology 24, 1053–1063.

Campiche S, L'Arnbert G, Tarradellas J and Becker-van Slooten K, 2007. Multigeneration effects of insect growth regulators on the springtail *Folsomia candida*. Ecotoxicology and Environmental Safety, 67, 180–189.

Ernst G, Kabouw P, Barth M, Marx MT, Frommholz U, Royer S, Friedrich S, 2016. Assessing the potential for intrinsic recovery in a Collembola two-generation study: possible implementation in a tiered soil risk assessment approach for plant protection products. Ecotoxicology, 25, 1–14.

Hornung E, Farkas S, Fischer E, 1998. Tests on the isopod *Porcellio scaber*. In: Løkke H, Van Gestel CAM. (Eds). Handbook of Soil Invertebrate Toxicity Tests.John Wiley & Sons, Chichester: 207–226.

Houx NWH, Dekker A, vanKammenPolman AMM and Ronday R, 1996. Acute toxicity test for terrestrial hazard assessment with exposure of *Folsomia candida* to pesticides in an aqueous medium. Archives of Environmental Contamination and Toxicology, 30, 9–14.

ISO (International Organisation for Standardization), 2005. Soil quality-Effects of pollutants on insect larvae (Oxythyrea funesta)-Determination of acute toxicity (ISO 20963).

ISO (International Organisation for Standardization), 2006. Soil quality-Effects of pollutants on juvenile land snails (Helicidae)-Determination of the effects on growth by soil contamination (ISO 15952).

ISO (International Organisation for Standardization), 2012. Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*) - Part 1: Determination of acute toxicity using artificial soil substrate (ISO 11268).

ISO (International Organisation for Standardization), 2010. Water quality - Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda) (ISO 10872).

ISO (International Organisation for Standardization), 2012 Soil quality-Effects of pollutants on earthworms-Part 2: Determination of effects on reproduction of *Eisenia fetida*/*Eisenia Andrei* (ISO 11268-2).

ISO (International Organisation for Standardization), 2014. Soil quality-Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants (ISO 11267).

ISO (International Organisation for Standardization), 2014. Soil quality-Effects of contaminants on Enchytraeidae (*Enchytraeus* sp.)-Determination of effects on reproduction (ISO 16387).

Jänsch S, Garcia M, Römbke J, 2005. Acute and chronic isopod testing using tropical *Porcellionides pruinosus* and three model pesticides. European Journal of Soil Biology, 41, 143–152.

Laskowski R, Pyza E, Maryański M, Niklińska M, 1998. Test on the centipede Lithobius mutabilis L. Koch 1852 (Chilopoda: Lithobiidae). In: Løkke H, Van Gestel CAM (eds) Handbook of Soil Invertebrate Toxicity Tests. John Wiley and Sons, Chichester, pp 181–196.

OECD (Organization for Economic Co-operation and Development), 1984. Earthworm Acute Toxicity tests (No. 207). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.

OECD (Organization for Economic Co-operation and Development), 2004. Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*) (No. 222). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.

OECD (Organization for Economic Co-operation and Development), 2004. Enchytraeid Reproduction Test (No 220). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.

OECD (Organization for Economic Co-operation and Development), 2008. Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer*) reproduction test in soil (No. 226). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.

OECD (Organization for Economic Co-operation and Development), 2009. Collembolan Reproduction Test in Soil (No. 232). OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.

Princz JI, Behan-Pelletier VM, Scroggins RP and Siciliano SD, 2010. Oribatid mites in soil toxicity testing-The use of Oppia Nitens (CL KOCK) as a new test species. Environmental Toxicology and Chemistry, 29, 971–979.

Rundgren S, Augustsson AK, 1998. Tests on the Enchytraeid Cognettia sphagnetorum (Vejdovsky) 1977. In: Løkke, H., Van Gestel, C.A.M. (Editors) Handbook of soil invertebrate toxicity tests. John Wiley and Sons, Chichester, United Kingdom. pp 73–94.

Schrader G, Metge K and Bahadir M, 1998. Importance of salt ions in ecotoxicological tests with soil arthropods. Applied Soil Ecology, 7, 189–193.

Tajovsky K, 1998. Test on the millipede Brachydesmus superus Latzel 1884. In: Løkke H and Van Gestel CAM (eds): Handbook of Soil Invertebrate Toxicity Test, pp 197–205. Chichester, UK: John Wiley and Sons Ltd.

Van Gestel CAM, Doornekamp A, 1998. Tests on the oribatid mite Platynothrus peltifer. In: Løkke H, van Gestel CAM, editors. Handbook of soil invertebrate toxicity tests. Chichester: Wiley; 1998. pp. 113–130.
Wiles JA and Frampton GK, 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. Pesticide Science, 47, 273–285.

Appendix G – ISO standards with potential relevance in soil microbiology

Year	Methods	ISO reference
1995	Water quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test	ISO 10712
1997	Soil quality – Determination of soil microbial biomass – Part 1: Substrate-induced respiration method	ISO 14240-1:1997
1997	Soil quality – Determination of soil microbial biomass – Part 2: Fumigation-extraction method	ISO 14240-2:1997
1997	Soil quality – Biological methods – Determination of nitrogen mineralisation and nitrification in soils and the influence of chemicals on these processes	ISO 14238:1997*
2002	Soil quality – Determination of abundance and activity of soil microflora using respiration curves	ISO 17155:2002*
2002	Soil quality – Laboratory methods for determination of microbial soil respiration	ISO 16072:2002
2004	Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation	ISO 15685:2004*
2005	Soil quality – Determination of dehydrogenase activity in soils – Part 1: Method using triphenyltetrazolium chloride (TTC)	ISO 23753-1:2005
2005	Soil quality – Determination of dehydrogenase activity in soils – Part 2: Method using iodotetrazolium chloride (INT)	ISO 23753-2:2005
2009	Soil quality – Effects of pollutants on mycorrhizal fungi – Spore germination test	ISO 10832:2009
2010	Soil quality – Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates	ISO 22939:2010
2010	Soil quality – Determination of soil microbial diversity – Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis	ISO 29843-1:2010
2011	Soil quality – Determination of soil microbial diversity – Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	ISO 29843-2:2011
2011	Soil quality – Method to directly extract DNA from soil samples	ISO 11063:2011
2016	Soil quality – Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil	ISO 17601:2016
2016	Soil quality – Simple laboratory assessments for characterising the denitrification in soil – Part 1: Soil denitrifying enzymes activities	ISO/CD 20131-1**
2016	Soil quality – Simple laboratory assessments for characterising the denitrification in soil – Part 2: Assessment of the capacity of soils to reduce N_2O	ISO/CD 20131-2**

*: Updated in 2012.
**: Under Development.

Appendix H – Comparison of sensitivity between *Folsomia candida* and *Hypoaspis aculeifer*

Chronic toxicity data on collembolan species (*Folsomia candida*, 28-day NOEC) and mites (*Hyapoaspis aculeifer*, 14-day NOEC) were checked and extracted, when available, from the list of endpoints included in the EFSA conclusions on the risk assessment of active substances published on the EFSA website in the period 2009–2015.

Data were available on both groups of organisms for 51 substances. For completeness, chronic toxicity data on earthworms (*Eisenia fetida*, 56-day NOEC) were also extracted.

The aim of the data collection and extraction was to evaluate the sensitivity of mites, compared to other standard in-soil organisms. The comparison was primarily done with *Folsomia candida*, being mites and collembolan grouped together as micro-arthropods. The toxicity data on mites were, however, also compared to those on *Eisenia fetida* to evaluate the position of mites compared to the other 2 standard species when looking at the sensitivity to PPPs.

The sensitivity ratio (R) between the endpoint for *Folsomia candida* and *Hypoaspis aculeifer* and between *Eisenia fetida* and the mite were calculated. When R was higher than 1, the mite species was more sensitive than either *Folsomia* or *Eisenia* spp. The results and discussion are reported in Section 9.

Table H.1: Chronic toxicity data on in-soil organisms as extracted from the EFSA conclusions on active substances

Test substance	Function of the substance	Endpoint *Folsomia candida* (28-day NOEC in mg a.s./kg soil)	Endpoint *Hypoaspis aculeifer* (14-day NOEC in mg a.s./kg soil)	Endpoint *Eisenia fetida* (56-day NOEC in mg a.s./kg soil)	*R* (*Folsomia*/ *Hypoaspis*)	*R* (*Eisenia*/ *Hypoaspis*)	Reference
Isoproturon	Herbicide	24.3	458.7	14	0.05	0.0305	EFSA (2015a)
Thifensulfuron methyl_metabolite INA4098	Herbicide	0.045	100	0.2	0.0005	0.002	EFSA (2015b)
Thifensulfuron methyl_metabolite INW8268	Herbicide	100	50	8	2	0.16	EFSA (2015b)
Famoxadone_metabolite IN-MN467	Fungicide	25	25	50	1	2	EFSA (2015c)
Famoxadone_metabolite IN-MN468	Fungicide	50	50	50	1	1	EFSA (2015c)
Iprovalicarb	Fungicide	1,000	1,000	64	1	0.064	EFSA (2015d)
Metalaxyl-M (formulation)	Fungicide	89	16.6	35.63	5.36	2.146	EFSA (2015e)
Tricyclazole (formulation)	Fungicide	32	16	5.2	2	0.325	EFSA (2015f)
Flupyradifurone (formulation)	Insecticide	1.44	170	1.5	0.01	0.009	EFSA (2015 g)
Florasulam metabolite_5-OH-florasulam	Herbicide	2.5	1.25	0.14	2	0.112	EFSA (2015 h)
Florasulam metabolie_DFP-ASTCA	Herbicide	10	10	0.03	1	0.003	EFSA (2015 h)
Florasulam metabolite_ASTCA	Herbicide	12.5	100	1	0.13	0.01	EFSA (2015 h)
Florasulam metabolite_TSA	Herbicide	50	50	10	1	0.2	EFSA (2015 h)
Triasulfuron_metabolite CGA 150829	Herbicide	100	100	30	1	0.3	EFSA (2015i)
Halauxiphen-methyl	Herbicide	500	12.5	5	40	0.4	EFSA (2014a)
Halauxiphen-methyl_metabolite X11449757	Herbicide	2.5	25	10	0.10	0.4	EFSA (2014a)
Flupyrsulfuron_metabolite IN-J0290	Herbicide	50	100	100	0.50	1	EFSA (2014b)
Flupyrsulfuron_metabolite IN-JV460	Herbicide	50	100	100	0.50	1	EFSA (2014b)
Flupyrsulfuron_metabolite IN-KC576	Herbicide	100	100	25	1	0.25	EFSA (2014b)
Flupyrsulfuron_metabolite IN-KT982	Herbicide	50	100	100	0.50	1	EFSA (2014b)
Flupyrsulfuron_metabolite IN-KV996	Herbicide	100	100	100	1	1	EFSA (2014b)
Flupyrsulfuron_metabolite IN-KY374	Herbicide	100	10	200	10	20	EFSA (2014b)
Esfenvalerate	Insecticide	0.4	2	0.55	0.20	0.275	EFSA (2014c)
2,4-D_metabolite 2,4-DCA	Herbicide	5	5	5	1	1	EFSA (2014d)
2,4-D_metabolite 2,4-DCP	Herbicide	0.625	2.5	5	0.25	2	EFSA (2014d)

Test substance	Function of the substance	Endpoint *Folsomia candida* (28-day NOEC in mg a.s./kg soil)	Endpoint *Hypoaspis aculeifer* (14-day NOEC in mg a.s./kg soil)	Endpoint *Eisenia fetida* (56-day NOEC in mg a.s./kg soil)	*R* (*Folsomia*/ *Hypoaspis*)	*R* (*Eisenia*/ *Hypoaspis*)	Reference
Prosulfuron_metabolite CGA 150829	Herbicide	0.225	100	8	0.00225	0.08	EFSA (2014e)
Pyridate (formulation)	Herbicide	250	250	No data	1	Not applicable	EFSA (2014f)
Ethametsulfuron-methyl	Herbicide	100	100	No data	1	Not applicable	EFSA (2014 g)
Sulfosulfuron (formulation)	Herbicide	1,000	1,000	No data	1	Not applicable	EFSA (2014 h)
Sulforsulfuron_metabolite sulfonyl biuret	Herbicide	0.0275	0.0275	0.0275	1	1	EFSA (2014 h)
Sulforsulfuron_metabolite sulfosulfuron guanidine	Herbicide	0.1	0.1	0.1	1	1	EFSA (2014 h)
Sulfoxaflor (formulation 1)	Insecticide	0.3204	12	0.09	0.03	0.0075	EFSA (2014i)
Sulfoxaflor (formulation 2)	Insecticide	0.08	3.125	0.08	0.03	0.0256	EFSA (2014i)
Sulfoxaflor_metabolite X11519540	Insecticide	10	10	10	1	1	EFSA (2014i)
Sulfoxaflor_metabolite X11579457	Insecticide	10	10	10	1	1	EFSA (2014i)
Lambda-Cyhalothrin (formulation)	Insecticide	2.73	4.67	No data	0.58	Not applicable	EFSA (2014j)
Metobromuron	Fungicide	23.66	23.66	No data	1	Not applicable	EFSA (2014k)
Tebuconazole	Fungicide	250	50	No data	5	Not applicable	EFSA (2014)l
Chromofenozide_metabolite M-010	Insecticide	1,000	1,000	No data	1	Not applicable	EFSA (2013a)
Metaflumizone (formulation)	Insecticide	110	27.6	119.74	3.99	4.338	EFSA (2013)EFSA (2014b)
Chlorantraniliprole	Insecticide	0.39	100	No data	0.0039	Not applicable	EFSA (2013c)
Fluopyram (formulation)	Fungicide	103.8	415	11.42	0.25	0.0275	EFSA (2013d)
Ametoctradin_metabolite M650F03	Fungicide	50	100	83.5	0.50	0.835	EFSA (2012a)
Ametoctradin_metabolite M650F04	Fungicide	100	100	100	1	1	EFSA (2012a)
Bixafen (formulation)	Fungicide	7.74	6.15	9.3	1.26	1.512	EFSA (2012b)
Penflufen (formulation)	Fungicide	115.55	246.5	16.5	0.47	0.067	EFSA (2012c)
Penflufen_metabolite M01	Fungicide	1,000	1,000	1,000	1	1.0000	EFSA (2012c)
Penflufen_metabolite M02	Fungicide	500	500	250	1	0.5	EFSA (2012c)
Sedaxane (formulation)	Fungicide	228	228	2.62	1	0.0115	EFSA (2012d)
Fluxapyroxad (formulation)	Fungicide	2.99	29.64	21.3	0.10	0.7186	EFSA (2012e)
Tebufenpyrad	Acaricide	6.25	200	0.17	0.03	0.0009	EFSA (2008)

References

EFSA (European Food Safety Authority), 2008. Conclusion on the peer review of the pesticide risk assessment of the active substance tebufenpyrad. EFSA Journal, 192, 99 pp.
EFSA (European Food Safety Authority), 2012a. Conclusion on the peer review of the pesticide risk assessment of the active substance ametoctradin (BAS 650 F). EFSA Journal, 10, 84 pp.
EFSA (European Food Safety Authority), 2012b. Conclusion on the peer review of the pesticide risk assessment of the active substance bixafen. EFSA Journal, 10, 87 pp.
EFSA (European Food Safety Authority), 2012c. Conclusion on the peer review of the pesticide risk assessment of the active substance penflufen. EFSA Journal, 10, 74 pp.
EFSA (European Food Safety Authority), 2012d. Conclusion on the peer review of the pesticide risk assessment of the active substance sedaxane. EFSA Journal, 10, 76 pp.
EFSA (European Food Safety Authority), 2012e. Conclusion on the peer review of the pesticide risk assessment of the active substance fluxapyroxad. EFSA Journal, 2012, 10, 99 pp.
EFSA (European Food Safety Authority), 2013a. Conclusion on the peer review of the pesticide risk assessment of the active substance chromofenozide. EFSA Journal, 11, 63 pp.
EFSA (European Food Safety Authority), 2013b. Conclusion on the peer review of the pesticide risk assessment of the active substance metaflumizone. EFSA Journal, 11, 98 pp.
EFSA (European Food Safety Authority), 2013c. Conclusion on the peer review of the pesticide risk assessment of the active substance chlorantraniliprole. EFSA Journal, 2013, 11, 107 pp.
EFSA (European Food Safety Authority), 2013d. Conclusion on the peer review of the pesticide risk assessment of the active substance fluopyram. EFSA Journal, 2013, 76 pp.
EFSA (European Food Safety Authority), 2014a. Conclusion on the peer review of the pesticide risk assessment of the active substance halauxifen-methyl (XDE-729 methyl). EFSA Journal, 2014, 93 pp.
EFSA (European Food Safety Authority), 2014b. Conclusion on the peer review of the pesticide risk assessment of the active substance flupyrsulfuron. EFSA journal, 12, 82 pp.
EFSA (European Food Safety Authority), 2014c. Conclusion on the peer review of the pesticide risk assessment of the active substance esfenvalerate. EFSA journal, 12, 125 pp.
EFSA (European Food Safety Authority), 2014d. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D. EFSA Journal, 12, 78 pp.
EFSA (European Food Safety Authority), 2014e. Conclusion on the peer review of the pesticide risk assessment of the active substance prosulfuron. EFSA journal, 2014, 12, 94 pp.
EFSA (European Food Safety Authority), 2014f. Conclusion on the peer review of the pesticide risk assessment of the active substance pyridate. EFSA Journal, 12, 84 pp.
EFSA (European Food Safety Authority), 2014 g. Conclusion on the peer review of the pesticide risk assessment of the active substance ethametsulfuron (evaluated variant ethametsulfuron-methyl). EFSA Journal, 2014, 94 pp.
EFSA (European Food Safety Authority), 2014 h. Conclusion on the peer review of the pesticide risk assessment of the active substance sulfosulfuron. EFSA Journal, 12, 79 pp.
EFSA (European Food Safety Authority), 2014i. Conclusion on the peer review of the pesticide risk assessment of the active substance sulfoxaflor. EFSA Journal, 12, 170 pp.
EFSA (European Food Safety Authority), 2014j. Conclusion on the peer review of the pesticide risk assessment of the active substance lambda-cyhalothrin. EFSA Journal, 12, 170 pp.
EFSA (European Food Safety Authority), 2014k. Conclusion on the peer review of the pesticide risk assessment of the active substance metobromuron. EFSA Journal, 12, 78 pp.
EFSA (European Food Safety Authority), 2014 l. Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole. EFSA Journal, 12, 98 pp.
EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance triasulfuron. EFSA Journal, 13, 78 pp.
EFSA (European Food Safety Authority), 2015a. Conclusion on the peer review of the pesticide risk assessment of the active substance isoproturon. EFSA Journal, 13, 99 pp.
EFSA (European Food Safety Authority), 2015b. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl. EFSA Journal, 13, 144 pp.
EFSA (European Food Safety Authority), 2015c. Conclusion on the peer review of the pesticide risk assessment of the active substance famoxadone. EFSA Journal, 13, 116 pp.
EFSA (European Food Safety Authority), 2015d. Conclusion on the peer review of the pesticide risk assessment of the active substance iprovalicarb. EFSA Journal, 13, 82 pp.
EFSA (European Food Safety Authority), 2015e. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal, 13, 105 pp.
EFSA (European Food Safety Authority), 2015f. Conclusion on the peer review of the pesticide risk assessment of the active substance tricyclazole. EFSA Journal, 13, 65 pp.

EFSA (European Food Safety Authority), 2015 g. Conclusion on the peer review of the pesticide risk assessment of the active substance flupyradifurone. EFSA Journal, 13, 101 pp.
EFSA (European Food Safety Authority), 2015 h. Conclusion on the peer review of the pesticide risk assessment of the active substance florasulam. EFSA Journal, 13, 138 pp.

Appendix I – Advantages and disadvantages of methods to study microbial genetic and functional diversity

(From: Nannipieri et al., 2003; Kirk et al., 2004; Malik et al., 2008; Rastogi and Sani, 2011; Fakruddin and Mannan, 2013; Rincon-Florez et al., 2013 (the list is not considered exhaustive))

	Methods	Brief description	Advantage	Disadvantage
Abundane				
	Plate counting	Estimation the number of viable cells present on a diluted soil suspension based on their ability to give rise to colonies under specific conditions of nutrient medium, temperature and time	• Easy • Fast • Cost effective	• 0.1% to 1% of the soil bacterial population can be cultured • Interdependency of different organisms upon each other • Growth conditions such as temperature, pH, and light and to the fact that some microbial species are cultivable only under certain physiological conditions impact of the growing medium on the colony formed.
	Most Probable Number (MPN)	Estimation of population size without counting cells or colonies. The technique uses a statistical approach in which successive dilutions are made to reach an extinction point. Replicates of each dilution are inoculated into a liquid growth medium and the pattern of positive or negative scores recorded. A statistical table is then used to determine the MPN of viable organisms in the original sample	• Easy • Fast • Cost effective	• 0.1% to 1% of the soil bacterial population can be cultured
	Fatty acid methyl ester analysis	This method provides information on the microbial community composition based on groupings of fatty acids. Fatty acids make up a relatively constant proportion of the cell biomass and signature fatty acids	• Culturing of microorganisms is not required • Direct extraction from soil • Follow specific organisms or communities	• If fungal spores are used, more material is needed • Can be influenced by external factors • Many fatty acids are common

	Methods	Brief description	Advantage	Disadvantage
		exist that can differentiate major taxonomic groups within a community. Therefore, a change in the fatty acid profile would represent a change in the microbial population.	• Useful information on the dynamics of viable bacteria • Reproducible	• To different microorganisms • Time consuming • Low number of samples can be treated at the same time
	Phospholipid Fatty Acid Analysis (PLFA)	The technique is based on the premise that phospholipids are rapidly degraded, therefore phospholipids remaining should belong to living organisms. Phospholipids derived from microbial cell membranes can be used to distinguish specific microbial taxa. These phospholipids contain unique fatty acids composed of different acyl chains and can be used as biomarkers for microbial groups. Changes in the phospholipid profiles are generally related to variation in the abundance of microbial groups	• Sensitive detection and accurate quantification of different microbial groups • Rapid and efficient • Useful information on the dynamics of viable bacteria • Reproducible	• Time consuming • Low number of samples can be treated at the same time
	Quantitative PCR (Q-PCR) or real-time PCR	It is a technique that collects amplification data while the PCR occurs. Q-PCR uses either intercalating fluorescent dyes such as SYBR green or fluorescent probes (TaqMan) to measure the accumulation of amplicons in real time during each cycle of the PCR. When the Q-PCR is coupled with a preceding reverse transcription (RT) reaction, it can be used to quantify gene expression template abundance	• Quick, accurate and highly sensitive method for sequence quantification that can also be used to quantify microbial groups • Relatively cheap and easy to implement • Specific amplification can be confirmed by melting curve analysis	• Can only be used for targeting of known sequences. • DNA impurities and artefacts may create false-positives or inhibit amplification
Diversity				
	Mol% Guanine+Cytosine	Methods for analysis of base distribution of DNA.It is based on the knowledge that microorganisms differ in their G + C content and that taxonomically related groups only differ between 3% and 5%. It is	• Not influenced by PCR biases • Includes all DNA extracted • Includes rare members of community	• Requires large quantities of DNA • Dependent on lysing and extraction efficiency • Coarse level of resolution

	Methods	Brief description	Advantage	Disadvantage
		considered a low resolution method as different taxonomic groups may share the same G + C range		• Not informative on diversity parameters, which are richness, evenness and composition.
	Nucleic acid reassociation and hybridisation	DNA reassociation is a measure of genetic complexity of the microbial community and has been used to estimate diversity. Total DNA is extracted from environmental samples, purified, denatured and allowed to re-anneal. Nucleic acid hybridisation uses specific probes. These hybridisation techniques can be done on extracted DNA or RNA, or *in situ*. Oligonucleotide or polynucleotide probes designed from known sequences ranging in specificity from domain to species can be tagged with markers at the 5'-end. The relative abundance may represent changes in the abundance in the population or changes in the activity and hence the amount of rRNA content	• Total DNA extracted • Not influenced by PCR • biases • Study DNA or RNA • Can be studied *in situ*	• Lack of sensitivity • Sequences need to be in high copy number to be detected • Dependent on lysing and extraction efficiency
	DNA microarrays and DNA hybridisation	DNA microarray is a miniaturised array of complementary DNA probes ($\sim$ 500–5000 nucleotides inlength) or oligonucleotides (15–70 bp) attached directly to a solid support, which permits simultaneous hybridisation of a large set of probes complementary to their corresponding DNA/RNA targets in a sample	• Powerful for rapid characterisation as a single array can contain 500–1000 different DNA array • Total DNA extracted • Not influenced by PCR biases • Thousands of genes can be analysed • If using genes or DNA fragments, increased specificity	• Only detect the most abundant species • Need to culture organisms • Only accurate in low diversity systems

	Methods	Brief description	Advantage	Disadvantage
	Clone library	This method consists in cloning PCR products and then sequence the individual gene fragments. The obtained sequences are then compared to known sequences in a database, such as GenBnk, Ribosomal Database Project (RBP), Greengenes, etc. Typically cloned sequences are assigned to phylum, class, order, family, subfamily or species at sequence similarity cut-off value of 80, 82, 90, 92, 94 or 97%, respectively.	• Current 'gold standard' for obtaining the greatest estimate of diversity	• Labor-intensive • Time-consuming • Cost factor • Typical clone libraries of 16S rDNA genes contain fewer than 1,000 sequences and therefore reveal only a small portion of the microbial diversity present in a sample.
	Denaturing and Temperature Gradient Gel Electrophoresis (DGGE and TGGE)	These methods are considered intermediate resolution techniques. They are able to separate mixtures of PCR products that are similar in length but differ in base pair composition. The PCR amplified DNA have usually a limited size of 500 bp.Separation of bands in both DGGE and TGGE depends on decreased electrophoretically mobility of partial melted double stranded DNA molecules in a gel of polyacrylamide containing a linear gradient of DNA denaturant (DGGE) or linear temperature gradient (TGGE).	• Rapid and simple • Single band can be extracted from acrylamide gel and sequenced or hybridisation	• Dependent on lysing and extraction efficiency • Different fingerprints can be generated from the same DNA mixture • One band can represent more than one species (co-migration) • Only detects dominant population representing 1% of the total community

	Methods	Brief description	Advantage	Disadvantage
	Single Strand Conformation Polymorphism (SSCP)	The technique relies on electrophoretic separation based on differences in DNA sequences is single strand conformation polymorphism (SSCP). Single-stranded DNA is separated on a polyacrylamide gel based on differences in mobility caused by their folded secondary structure. When DNA fragments are of equal size and no denaturant is present, folding and hence mobility will be dependent on the DNA sequences.	• Same as DGGE/TGGE • No GC clamp • No gradient	• PCR biases • Some ssDNA can form more than one stable conformation
	Restriction Fragment Length Polymorphism (RFLP)	This method relies on DNA polymorphisms. With this method electrophoresed digests are blotted from agarose gels onto nitro-cellulose or nylon membranes and hybridised with appropriate probes prepared from cloned DNA segments of related organisms.	• Detect structural changes in microbial community	• PCR biases • Some ssDNA can form more than one stable conformation
	Terminal Restriction Fragment Length Polymorphism (T-RFLP)	It follows the same principle as RFLP except that one PCR primer is labelled with a fluorescent dye. Resulting PCR products are digested with restriction enzyme and terminal restriction fragments and then separated on an automated DNA sequencer	• Simpler banding patterns than RFLP • Can be automated large number of samples • Highly reproducible • Ability to compare differences between microbial communities	• Dependent on extraction and lysing efficiency • PCR biases • Type of Taq can increase variability • Choice of restriction enzymes will influence community fingerprint

	Methods	Brief description	Advantage	Disadvantage
	Ribosomal Intergenic Spacer Analysis (RISA)/ Automated Ribosomal Intergenic Spacer Analysis (ARISA)/ Amplified Ribosomal DNA Restriction Analysis (ARDRA)	RISA, ARISA and ARDRA provide ribosomal-based fingerprinting of the microbial community. In RISA and ARISA, the intergenic spacer (IGS) region between the 16S and 23S ribosomal subunits is amplified by PCR, denatured and separated on a polyacrylamide gel under denaturing conditions. This region may encode tRNAs and is useful for differentiating between bacterial strains and closely related species because of heterogeneity of the IGS length and sequence. Sequence polymorphisms are detected by silver staining in RISA. In ARISA, fluorescently labelled forward primer is detected automatically. In ARDRA, PCR amplified 16S rRNA fragments are digested or cut at specific sites with restriction enzymes and the resulting digest separated by gel electrophoresis.	• Highly reproducible community profiles	• Requires large quantities of DNA (for RISA) • PCR biases
	Random amplified Polymorphic DNA (RAPD)	The technique uses PCR amplification with short (usually 10 nucleotides) primers, which anneal randomly at multiple sites on the genomic DNA under low annealing temperature	• Suitable for unknown genomes • Requires low quantities of DNA. • Efficient, fast and low cost	• Low reproducibility • Sensitive to reaction conditions

	Methods	Brief description	Advantage	Disadvantage
	FISH	It is used to enable *in situ* phylogenetic identification and enumeration of individual microbial cells by whole cell hybridisation with oligonucleotide probes. A fluorescent molecule or fluorochrome is conjugated with an oligonucleotide probe. In microbiology, 16S rRNA is generally used as a probe due to its genetic stability and high copy number. The fluorescent probe binds to a complementary sequence that can therefore be detected using fluorescence microscopy.	• Allows detection and spatial distribution of more than one samples at the same time	• Autofluorescence of microorganisms • Accuracy and reliability is highly dependent on specificity of probe(s)
	Whole microbial Genome sequencing	Whole microbial genomes are sequenced using a shotgun cloning method that involves (i) extraction of DNS from pure cultures, (ii) random fragmentation of obtained genomic DNS into small fragments of ~ 2 kb, (iii) ligation and cloning of DNA fragments. Once the sequences are obtained, they are aligned and assembled into finished sequences using specialised computer programs.	• Rapid method to assess biodiversity and abundance of many species/ organisational taxonomic units simultaneously and at a considerable depth compared to the methods that have been available so far	• Relatively expensive • Replication and statistical analysis are essential • Computational intensive • Challenging in terms of data analysis
	Next Generation Sequencing (metagenomics)	Metagenomics is defined as the functional and sequence-based analysis of the collective microbial genomes that are contained in an environmental sample. The most widely used platforms for massive parallel sequencing for assessing soil microbial diversity are Roche 454 Genome Sequencer, Hi Seq 2000 and AB SOLiDTM System	• Biodiversity can be studied in more detail • Captures polymorphism in microbial communities • Reveals the presence of thousands of microbial genomes simultaneously – provides information about the functions of microbial communities in a given environment	• High cost • Data analysis is challenging and time-consuming • Difficult to use for low-abundance communities. • The high biodiversity in soil leads to many incomplete genomes – current sequencing methods and computing power still in its infancy to the high biodiversity in soil

	Methods	Brief description	Advantage	Disadvantage
Activity				
	Enzymatic activity assays and other biochemical assays (e.g. N-transformation test)	Dehydrogenase activity is one of the enzymatic assays which can be carried out to investigate the effects of pesticides on a specific function of microbial community. It represents the intracellular flux of electrons to O_2 and is due to the activity of several intracellular enzymes catalysing the transfer of hydrogen and electrons from one compound to another. Nitrification rate is the ultimate degradation by microorganisms of nitrogen-containing organic matter, via the process of ammonification and nitrification, to the respective inorganic end-product nitrate.	• Low-cost • Easy and fast method to measure microbial activity for soil samples	• The enzymatic assay do not distinguish the contribution of intracellular from extracellular and stabilised enzyme activities
	Fluorescein diacetate (FDA)	FDA is hydrolysed by free exoenzymes and membrane-bound enzymes that convert the colourless FDA in a coloured fluorescein. Fluorescein can then be quantified by spectrophotometry at 490 nm wavelength	• Low-cost • Easy and fast method to measure microbial activity for soil samples	• The measurement of soil microbes by FDA can be contaminated by external sources, e.g. plant matter • It is a measurement of the contribution of several enzymes such as non-specific esterases, proteases and lipases
	Stable Isotope Probing (SIP)	The technique allows the characterisation or identification of microbial population actively involved in specific metabolic processes in the environment. It involves the incorporation of stable isotope labelled substrates into cellular biomarkers that can be used to identify organisms assimilating the substrate	• Provides evidence on the function • of microorganisms in a controlled • experimental setup • Directly link microbial phylogeny with function	• Incubation and cycling of the stable • isotope might cause biases within the • microbial communities • Lack sensitivity • Enrichment bias may not reflect substrate metabolism in the environment

	Methods	Brief description	Advantage	Disadvantage
	Community level physiological profiling (CLPP)/Sole-Carbon-Source Utilisation (SCSU) Pattern (BIOLOG®)	SCSU or CLPP, also known as Biolog, examines the functional capabilities of the microbial population, and the resulting data can be analysed using multivariate techniques to compare metabolic capabilities of communities. It uses a commercially available 96-well microtitre plate to assess the potential functional diversity of the bacterial population through sole source carbon utilisation (SSCU) patterns. Gram-negative (GN) and Gram-positive (GP) plates are available from BIOLOG®	• Fast • Highly reproducible • Relatively inexpensive • Able to differentiate microbial communities • Generates large amount of data • Option of using bacterial, fungal plates or site specific carbon sources (Biolog)	• Only represents culturable fraction of community • Favours fast growing organisms (then contribution of fungi could not be measured as they usually grow slowly) • Only represents those organisms capable of utilising available carbon sources • Potential metabolic diversity, not *in situ* diversity • Sensitive to inoculum density • Reproducible results can only be obtained if replicates contain identical community profiles
	Functional Gene Arrays (FGA) (RNA-based)	Functional Gene Array identifies or measure genes encoding key enzymes in a metabolic process by measuring mRNA	• Analyses a vast amount of genetic information simultaneously	• Requires the construction of an array and access to a scanner • Issues with specificity/cross hybridisation • Requires normalisation • Insufficient sensitivity and reproducibility can be problematic • Limited by the presence of probes on the array • Issues with RNA extraction from soil

	Methods	Brief description	Advantage	Disadvantage
	Generation Sequencing (Metatranscrip tomics)	In a metatranscriptomic study total RNA is first isolated from the sample and structural RNAs are then removed to enrich for mRNA, which is then reverse transcribed into cDNA subject to DNA sequencing using next generation sequencing (NGS) platforms (See metagenomic description). Metatranscriptomic data indicate which of the genes encoded in a metagenome are actually transcribed, and which of the potential metabolic pathways are active (and the level of their activities) on the basis of their transcriptions within a microbial community under certain environmental condition. Metatranscriptomic sequences can be assembled into transcripts, each encoding one or more genes that are transcribed together (in the same direction). In the latter case (known as operons), the intergenic regions between coding genes are relatively short	• Allows rRNA and/or mRNA • Profiling and quantification without • Prior knowledge of sequence	• Many issues with isolation of RNA • from soil • mRNA isolation and often amplification are required for gene expression analyses • Current sequencing methods, databases and computing power are not sufficient yet to cover the high biodiversity in soil.

Abundance
Diversity
Activity

References

Fakruddin M and Mannan KSB, 2013. Methods for Analyzing Diversity of Microbial Communities in Natural Environments. Ceylon Journal of Science, 42, 19–33.

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Kironomos JN, Lee H and Trevors JT, 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods, 58, 169–188.

Malik S, Beer M, Megharaj M and Naidu R, 2008. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environment International, 34, 265–276.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G and Renella G, 2003. Microbial diversity and soil functions. European Journal of Soil Science, 54, 655–670.

Rastogi G and Sani RK, 2011. Molecular Techniques to Assess Microbial Community Structure, Function, and Dynamics in the Environment. In: Microbes and Microbial Technology- Agricultural and Environmental Applications. Eds Ahmad I, Ahmad F and Pichtel J. Springer Science and Business Media LLC, 29–57.

Rincon-Florez VA, Carvalhais LC and Schenk PM, 2013. Culture-Independent Molecular Tools for Soil and Rhizosphere Microbiology. Diversity, 5, 581–612.

Appendix J – Evaluation of the ECPA proposal to evaluate recovery in single species multigeneration studies with *F. candida*

ECPA proposed adapting the multigeneration study with Collembola (Campiche et al., 2007) to assess the internal recovery of Collembola following PPP application (please refer to Ernst et al., 2016).

The study design consists in principle of two subsequent chronic tests with *Folsomia candida*. The reproductive success of the collembolan exposed to PPP is assessed in an artificial soil matrix according to guideline OECD 232 (OECD, 2009). The whole study according to the new design lasts 70 days, the single experiments 28 days each. The artificial soil employed in the tests is contaminated prior to the first test, and is therefore slightly 'aged' at the start of the second chronic tests (42 days). Between the two chronic tests, 2 weeks are foreseen to rear and synchronise the retrieved organisms from the first test and to select the starting animals for the second tests. The endpoints given at test termination concern survival and reproductive success of *F. candida* in the first chronic test and of *F. candida* F1 generation in the second test with 'aged residues', respectively.

Two main issues need to be discussed with regard to the appropriateness of the study design to assess recovery:

1) Test species that has been chosen to address the risk to in-soil organisms in a higher tier approach

The chosen collembolan *Folsomia candida* is a soil dwelling organisms, generally employed in ecotoxicological laboratory testing. It has been shown to be fairly sensitive to toxicants, is easily reared and has a short reproductive cycle (21 days) with a high number of offspring per cycle (e.g. (Krogh, 2008). In our opinion, the life history traits displayed by *F. candida* might not be representative of ecologically sensitive, vulnerable species of the in-soil organisms' community in the field. Such vulnerable species (e.g. species with long life cycles) are, however, those that should be particularly considered when addressing long-term effects on non-target organisms communities after initial effects due to PPPs use. Please refer for further details on which species' traits should be addressed when considering recovery and/or recolonisation processes to EFSA (2014).

2) Consideration of the reproductive success of adults

The test design does not assess the reproductive success of the first adult generation after 28 days. Since these animals contribute further to the population size development and are also still exposed to the PPPs, it cannot be excluded that there are effects on collembolan populations in the long term solely because the second reproduction test delivers toxicity thresholds above the acceptability criterion.

Moreover, as discussed previously, all (experimental or modelling) approaches for assessing the recovery of in-soil organisms from use of a single PPP need to account for multiple stress caused by normal agricultural practice (e.g. sequential use of different pesticides) that might hinder recovery. This is not considered in the proposed assessment.

In the publication Ernst et al. (2016), it is stated that this test is also intended to be used to assess whether a substance loses its toxicity in soil fast or slowly. To check its usefulness for that purpose, the results of the 2-generation study are compared with higher tier TME and field study data, which act as a reference. Such a comparison is done for the substances lindane and chlorpyrifos-methyl. Whereas for chlorpyrifos-methyl, both tier 1 and 2-generation TERs are based on NOECs, for Lindane, the tier 1 TER is based on EC_{10} and the intermediate TER on NOEC. Comparable TER values for Lindane both based on NOECs would be 0.0625 and 0.16 for tier 1 and 2-generation, respectively. An intermediate tier (as this multigeneration study proposed by Ernst et al., 2016) would only be useful if results correlate better with surrogate reference tier results than first tier results, i.e. reduce the margin of safety more strongly for substances, for which tier 1 gives a higher margin of safety. Even though for chlorpyrifos-methyl the margin of safety based on TME NOECs is higher than for Lindane, the 2-generation data reduces the margin of safety for both substances to the same extent (factor of 2.5). Therefore based on the data presented by Ernst et al. (2016) it is not shown that the 2-generation study improves the prediction of field effects. It should be also noted that substances that degrade quickly are often applied multiple times. This is most likely the case, e.g. for the fungicides that lose their toxicity towards collembola in the two generation study quickly (cited case studies 4 and 11). Since the presented study considers only 1 application it seems likely that this will introduce a bias, in

the risk assessment, especially for less persistent substances that are applied multiple times. This would be therefore another important issue to be considered when further evaluating the appropriateness of the suggested intermediate tier approach.

Concluding, the outcome of the *F. candida* two generation test (Ernst et al., 2016) may not be fully appropriate to address the persistence of effects and the recovery of collembola.

References

Campiche S, L'Ambert G, Tarradellas J and Becker-van Slooten K, 2007. Multigeneration effects of insect growth regulators on the springtail *Folsomia Candida*. Ecotoxicology and Environmental Safety, 67, 180–189.

EFSA (European Food Safety Authority), 2014. EFSA's 19th Scientific Colloquium on Biodiversity as protection goal in environmental risk assessment for EU agro-ecosystems. EFSA Supporting Publication, doi:10.2903/sp.efsa.2014.EN-583.

Ernst G, Kabouw P, Barth M, Marx MT, Frommholz U, Royer S, Friedrich S, 2016. Assessing the potential for intrinsic recovery in a Collembola two-generation study: possible implementation in a tiered soil risk assessment approach for plant protection products. Ecotoxicology, 25, 1–14.

Appendix K – Semifield and field test systems

Table K.1: Potential (semi)field approaches to the risk assessment of plant protection products (PPP) towards non-target organisms (partly adapted from Brown et al. (2008) Schaeffer et al. (2010) with some additions)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Semifield studies					
Soil microorganisms and soil mesofauna	TME Species should taxonomic groups that lower tier risk assessments have identified as being of concern. Preferably a naturally developed soil community with organisms such as arthropods, nematodes and/or oligochaetes. Abundant species like oribatids and predatory mites, as well as collembolans should be present in adequate number. Organisms may be added. TMEs may be considered for studying earthworm populations except for large vertical burrowers (anecics) Natural soil cores including soil organisms	Diversity and abundance NOEC, EC_x expressed in mg/kg soil	Plateau concentration and or total annual application is applied on the soil of the TMEs (in order not to disturb the community). One year or more depending on the aim of the study (e.g. recovery of univoltine species) Concentration in soil is measured		Schäffer et al. (2010)
Earthworms *Dendrobaena octaedra,* *E. fetida*	A forest soil microcosm (forest litter)was used to characterise pesticide toxicity to earthworms Enables risk in specific circumstances to be evaluated. In this case, effects in forest areas with thick litter and high organic matter was evaluated	Burrowing time, weight change and cocoon production survival, reproduction		*E. fetida* did not thrive in this system, indicating that this methods may not be applicable for all earthworm species	Addison and Holmes (1995)
Earthworms *A. trapezoids*	Enclosures were made from PVC pipes. Earthworms were added and then treatments made. Thirty-eight days after treatment numbers of earthworms and cocoons assessed	Survival, number of cocoons		Smaller system that may enable dose rates to be assessed. *A. trapezoides* is a shallow-burrowing earthworm, and so may not appropriately model the susceptibility or exposure of deeper burrowing worms	Choo and Baker (1998)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworm *Aporrectodea caliginosa*	Microcosm system, constructed of using stainless steel enclosures 20 cm deep and 12 cm in diameter (5 replicates per treatment). Natural soil from the study area was used, microcosms were taken to the laboratory. Acetylcholinesterase inhibition in earthworms was shown to strongly correlate with biomass changes	Biomass, cholinesterase inhibition	Chlorpyrifos was applied to the soil surface of the microcosm	Low natural earthworm densities may confound results	Reinecke and Reinecke (2007)
Soil organisms involved in organic matter breakdown	Litter bags containing dried OM (wheat straw) buried in field soil; determination of ash-free dry weight of straw in litter bags after 1, 3 and 6 months after burying bags (or further sampling up to 12 months if 60% mass loss in control is not reached)	Mass loss of litter bags in treatment compared to mass loss of litter bags in control endpoint expressed as % effect (mass loss)	Plateau concentration + annual cumulative application rate (see dosing method) Concentration in soil measured immediately after application		Römbke et al. (2003)
Soil mesofauna enchytraeids, mites and nematodes	Field study aimed at the structure of the mesofauna community. Plots 3 × 7 m. Control, treatment and toxic reference (benomyl + chlorpyrifos), 6 replicates. Sampling 2 days before and 1, 3, 6 and 12 months after application	Abundance of enchytraeids, mites and nematodes. % difference with control	2 applications with test item; concentration in soil was measured	Mites appear to be less sensitive, even to the toxic reference	Römbke et al. (2009)
Soil microorganism communities Invertebrates: *Pelodera strongyloides,* Enchytraeidae	Microcosm comprised of bean plants *P. vulgaris,* phytophagous organisms, soil bacteria, fungi and microinvertebrates	Assessed endpoints include soil parameters, cellular indicators, indicators at the organism, population and community level		The assessment of multiple taxa at different trophic levels utilising cellular to community endpoints provides a thorough accounting of potential effects. Methodology requires validation. Ability to use in risk assessment needs to be confirmed	Motheswagner et al. (1992)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworm *Aporrectodea tuberculata*	Micrososms were filled with field collected soil, planted with wheat seedlings, and three earthworms were added to each system Treatment regime was designed to mimic natural spray events, adding realism to microcosm studies, and endpoints were selected to give insight concerning ecosystem processes	Microbial biomass, litter decomposition, enzyme activity, bait lamina tests, nutrient leaching measurements were taken periodically, and abundances were quantified at the end	Pesticide was measured	Collection and containment of natural soil communities may cause problems with non-homogenous replicate communities	Edwards et al. (1998)
Earthworm *Aporrectodea trapezoides*	Mesocosm study, including the use of mesh bags with organic matter buried in 15 cm deep units with one earthworm. Toxicity thresholds produced using this methodology was very similar to those produced using other methods, indicating that this method has been verified	Earthworm survival, growth, body accumulation, organic matter decomposition, substrate induced respiration, soil urease activity and total nematode numbers		Use of a single earthworm could limit statistical analysis. Soil chemistry, including pH, may alter sensitivity of soil invertebrate to plant protection products.	Bogomolov et al. (1996)
Nematodes Earthworms *L. rubellus*	Microcosm (using soil cores taken from field), in which three *L. rubellus* were added to each enclosure. The use of soil cores allows for the collection and analysis of leachate (PPP and nutrients)	Nematode populations, bait lamina test, growth of earthworms, nitrate concentration	Field soil. Dosages mixed through the soil. Concentration measured after application	Methods for interpretation and extrapolation of microcosm results for use in risk assessments have not yet been developed	Burrows and Edwards (2002)
Soil community	Reference describes methodology for the automated collection of soil core leachate, irrigation, and analysis of CO_2 production. Automation streamlines the process of conducting soil microcosm studies			Unexpected differences detected in CO_2 production of soil core microcosms were not explained	Hantschel et al. (1994)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Earthworms *E. fetida,* Enchytraeidae *Enchytraeus albidus*	Both laboratory and field studies were conducted to determine the efficacy of using lab experiments to predict impacts in the field Soil cores were exposed in greenhouse conditions, while field communities were subjected to overspray. Inhomogeneity of earthworm distribution in field was realistically reflected by the TMEs			The authors conclude that the abundance and biomass of earthworms are suitable endpoints for assessment of chemicals within TME's but at sites where abundance is low data interpretation may be difficult. Predictability of biomass results derived from TME's is restricted if the number of large earthworms, such as *L. terrestris* or *L. rubellus*, is high	Römbke et al. (2004)
Collembola, Astigmata, Cryptostigmata, Mesostigmata, Prostigmata Microarthropod	TME: Soil cores were collected from multiple fields, irrigated, acclimated for 2–4 weeks, and treated with compound. Sampling was conducted at weeks 1, 4, 8 and 16 following exposure. Conclusions in TME mirrored those in the field study and thus predictive value of TME is illustrated. Large variations in both Collembola and mite communities			Differences in the vegetation in the TMEs in the four countries possibly caused variation in soil moisture, which may have affected soil microarthropod communities independently of exposure	Koolhaas et al. (2004)
Nematodes	Methodology was developed concerning the impact of pesticides on soil dwelling nematodes as a part of the ring-testing of Terrestrial Model Validation of TME since field studies showed the same responses to exposure	Trophic structure, population		High variability of data may conceal effects and increase the likelihood of misinterpretation	Moser et al. (2004)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Enchytraeidae, earthworms decomposition	TME to measure the breakdown of organic matter. The breakdown of cellulose inserted into a soil column or on the soil surface. Faunal feeding was measured with a bait lamina method. Effects on organic matter decomposition were the same in the TME and the field study and showed a dose response relationship			The feeding activity of the soil fauna showed a large variability.	Forster et al. (2004)
Soil microbial community	A coupled set of experiments (one laboratory and one field) were conducted to describe the impact of a pesticide on soil microorganisms. Various microbial parameters measured. Comparisons on data variability also revealed the absence of significant differences between experiments in all parameters in most cases, indicating that TMEs were able to represent the spatial variability found in the field Measured responses to the model chemical in TMEs were similar to the field study			Soil moisture lead to some of the variability in microbial parameters.	Sousa et al. (2004)
Nutrient cycling	TME impact of PPP on soil nutrient cycling. Soil and leachate ammonium and nitrite concentrations were measured following application. Field data showed similar patterns in nutrient levels and thus the TME's predictive value was confirmed			Variability in moisture or invertebrate activity may confound results. Because soil invertebrates are not homogenously distributed in field soils, columns may contain significantly different community structures or abundances	Van Gestel et al. (2004)

Organism	Test design	Endpoints	Exposure	Remarks	Reference
Soil-organisms feeding on organic matter	Small apertures in bait-lamina sticks, filled with a mixture of cellulose (70%), finely ground wheat bran (25%), and activated carbon (5%). Sticks are vertically put into the uppermost soil layer (about 8 cm) for a period of about 3 weeks (temperate regions) to 4 days (tropics)	Number of empty apertures (i.e. areas from which the bait material has been removed) as well as their vertical distribution along the strip	Not specified; i.e. normal application pattern and rates	Limited experience for agricultural sites	ISO, 2016 (18311)
Field studies					
Earthworm (natural occurring species)	Field study. Increasingly a dose response design is used, and substance is measured in the soil	Diversity, abundance and biomass. 1, 4–6 and 12 months after application. Expressed as kg/ha (active substance), nominal	Duration depends on characteristics of test substance, usually 1 year Field application according to GAP		ISO (2014) (11268–3)

References

Addison JA and Holmes SB, 1995. Comparison of forest soil microcosm and acute toxicity studies for determining effects of fenitrothion of earthworms. Ecotoxicology and Environmental Safety, 30, 127–133.

Bogomolov DM, Chen SK, Parmelee RW, Subler S and Edwards CA, 1996. An ecosystem approach to soil toxicity testing: A study of copper contamination in laboratory soil microcosms. Applied Soil Ecology, 4, 95–105.

Brown K, Tomlinson J, Duncan J and Hinchcliffe A, 2008. Critical comparison of available and potential higher tier testing approaches for the risk assessment of plant protection products, considering at least field and semi-field experimental designs, extrapolation from dose-response relationships, and increased dosages (aquatic and terrestrial). EFSA supporting publication, DOI: 10.2903/sp.efsa.2009.EN-16. http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2009.EN-16/abstract

Burrows LA and Edwards CA, 2002. The use of integrated soil microcosms to predict effects of pesticides on soil ecosystems. European Journal of Soil Biology, 38, 245–249.

Burrows LA and Edwards CA, 2004. The use of integrated soil microcosms to assess the impact of carbendazim on soil ecosystems. Ecotoxicology, 13, 143–161.

Choo LPD and Baker GH, 1998. Influence of four commonly used pesticides on the survival, growth, and reproduction of the earthworm *Aporrectodea trapezoides* (Lumbricidae). Australian Journal of Agricultural Research, 49, 1297–1303.

Edwards CA, Knacker T and Pokarzhevskii A, 1998. The prediction of the fate and effects of pesticides in the environment using tiered laboratory soil microcosms. Pests and Diseases, 267–272.

Forster B, Van Gestel CAM, Koolhaas JE, Nentwig G, Rodrigues JML, Sousa JP, Jones SE and Knacker T, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on organic matter breakdown and soil fauna feeding activity. Ecotoxicology, 13, 129–141.

Hantschel RE, Flessa H and Beese F, 1994. An Automated Microcosm System for Studying Soil Ecological Processes. Soil Science Society of America Journal, 58, 401–404.

ISO (International Organisation for Standardization), 2014. Effects of pollutants on earthworms-Part 3: Guidance on the determination of effects in field situations (ISO 11268-3). Revises ISO 11268-3 (1999).

ISO (International Organisation for Standardization), 2016. Soil quality - Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test. ISO 18311.

Koolhaas JE, Van Gestel CAM, Römbke J, Soares A and Jones SE, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on soil microarthropod communities. Ecotoxicology, 13, 75–88.

Moser T, Schallnass HJ, Jones SE, Van Gestel CAM, Koolhaas JE, Rodrigues JML and Römbke J, 2004. Ring-testing and field-validation of a terrestrial model ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on nematodes. Ecotoxicology, 13, 61–74.

Motheswagner U, Reitze HK and Seitz KA, 1992. Terrestrial Multispecies Toxicity testing. 1. Description of the Multispecies Assemblage. Chemosphere, 24, 1653–1667.

Reinecke SA and Reinecke AJ, 2007. Biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms. Ecotoxicology and Environmental Safety, 66, 92–101.

Römbke J, Heimbach F, Hoy S, Kula C, Scott-Fordsmand J, Sousa P, Stephenson G, Weeks J, 2003. Effects of Plant Protection Products on Functional Endpoints in Soil (EPFES). SETAC, Lisbon, 24–26 April 2002. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, Florida, USA.

Römbke J, Van Gestel CAM, Jones SE, Koolhaas JE, Rodrigues JML and Moser T, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on earthworms. Ecotoxicology, 13, 105–118.

Römbke, J., Schmelz, R.M., Knäbe, S. 2009: Field studies for the assessment of pesticides with soil mesofauna, in particular enchytraeids, mites, and nematodes: Design and first results. Soil Organisms, Volume 81 (2), 237–264.

Schäffer A, van den Brink P, Heimbach F, Hoy SP, de Jong F, Roembke J, Sousa JP and Ross-Nickoll M, 2008. Semi-field methods are a useful tool for the environmental risk assessment of pesticides in soil - Developed on the discussions at the SETAC workshop PERAS, Coimbra, Portugal, October 2007. Environmental Science and Pollution Research, 15, 176–177.

Schäffer A, van den Brink P, Heimbach F, Hoy SP, de Jong F, Römbke J, Ross-Nickoll M and Sousa JP, 2010. Semi-field methods for the environmental risk assessment of pesticides in soil. Eds Schäffer A, Brink PJvd, Heimbach F, Hoy SP, Jong FMWd, Römbke J, Ross-Nickoll M and Sousa JP. CRC Press, Boca Raton, FL, 105 pp.

Sousa JP, Rodrigues JML, Loureiro S, Soares A, Jones SE, Forster B and Van Gestel CAM, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on soil microbial parameters. Ecotoxicology, 13, 43–60.

Van Gestel CAM, Koolhaas JE, Schallnass HJ, Rodrigues JML and Jones SE, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) - An instrument for testing potentially harmful substances: Effects of carbendazim on nutrient cycling. Ecotoxicology, 13, 119–128.

Appendix L – Uncertainty

Background

Uncertainty was identified (EFSA Scientific Committee, 2013) as a priority topic: 'guidance should be developed on how to characterise, document and explain uncertainties in all steps for human and environmental risk assessment'. A working group was established and the terms of reference stated that the guidance should develop 'a harmonised framework applicable to all relevant working areas of EFSA'. The draft guidance will soon go out for public consultation and states that:

> 'The Scientific Committee considers that all EFSA scientific assessments must include consideration of uncertainties. Therefore the application of this guidance document is unconditional for EFSA. For reasons of transparency and in line with EFSA (2006), the assessments must say what uncertainties have been identified and what is their impact on the overall assessment outcome. This must be reported clearly and unambiguously'.

Purpose of appendix

What are the implications of the SC guidance for an opinion on science underlying a future guidance document?

- It should consider uncertainties in the underlying science and make an assessment of their potential to influence risk assessments.
- It should consider any additional uncertainties which need to be considered when developing the guidance.

It should possibly also make an initial evaluation of uncertainties which are likely to affect use of the guidance, distinguishing between those which are likely to be covered by assessment factors (AFs) and those which are not.

Main Uncertainties identified by the Working Group in the risk assessment of in-soil organisms:

- Representativeness of test species
- Temporal dynamics of effects
- Interspecies variability
- Are the Assessment Factors appropriate?
- Extrapolation Lab-to-field
- Soil variability
- Field exposure dynamics
- Linking test effects to specific protection goals
- Recovery
- Matching up effects and exposure estimates
- Power of higher tier tests
    - number of replicates required
    - field size/recolonisation issues
- Are metabolites covered?

References

EFSA (European Food Safety Authority), 2006. Transparency in risk assessment carried out by EFSA: 2604 Guidance document on procedural aspects. EFSA Journal 2006;353, 1–16.

EFSA Scientific Committee, 2013. Scientific Opinion on Priority topics for the development of risk assessment guidance by EFSA's Scientific Committee EFSA Journal 2013;11, 20 pp.